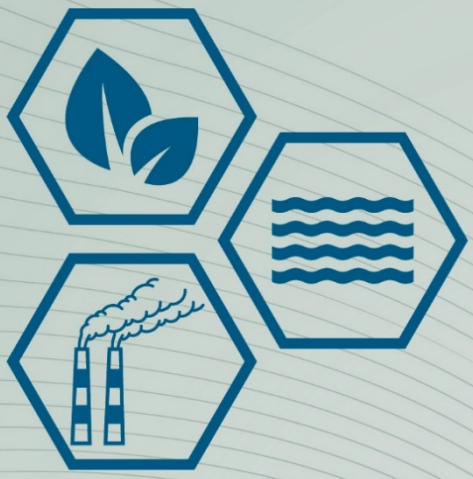

Toxicological Profile for 2,4-Dichlorophenoxyacetic Acid (2,4-D)

Draft for Public Comment

April 2017

ATSDR
U.S. Department of Health and Human Services
Agency for Toxic Substances and Disease Registry

CS274127-A

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UPDATE STATEMENT

Toxicological profiles are revised and republished as necessary. For information regarding the update status of previously released profiles, contact ATSDR at:

Agency for Toxic Substances and Disease Registry
Division of Toxicology and Human Health Sciences
Environmental Toxicology Branch
1600 Clifton Road NE
Mailstop F-57
Atlanta, Georgia 30329-4027

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FOREWORD

This toxicological profile is prepared in accordance with guidelines developed by the Agency for Toxic Substances and Disease Registry (ATSDR) and the Environmental Protection Agency (EPA). The original guidelines were published in the *Federal Register* on April 17, 1987. Each profile will be revised and republished as necessary.

The ATSDR toxicological profile succinctly characterizes the toxicologic and adverse health effects information for these toxic substances described therein. Each peer-reviewed profile identifies and reviews the key literature that describes a substance's toxicologic properties. Other pertinent literature is also presented, but is described in less detail than the key studies. The profile is not intended to be an exhaustive document; however, more comprehensive sources of specialty information are referenced.

The focus of the profiles is on health and toxicologic information; therefore, each toxicological profile begins with a public health statement that describes, in nontechnical language, a substance's relevant toxicological properties. Following the public health statement is information concerning levels of significant human exposure and, where known, significant health effects. The adequacy of information to determine a substance's health effects is described in a health effects summary. Data needs that are of significance to the protection of public health are identified by ATSDR and EPA.

Each profile includes the following:

(A) The examination, summary, and interpretation of available toxicologic information and epidemiologic evaluations on a toxic substance to ascertain the levels of significant human exposure for the substance and the associated acute, subacute, and chronic health effects;

(B) A determination of whether adequate information on the health effects of each substance is available or in the process of development to determine levels of exposure that present a significant risk to human health of acute, subacute, and chronic health effects; and

(C) Where appropriate, identification of toxicologic testing needed to identify the types or levels of exposure that may present significant risk of adverse health effects in humans.

The principal audiences for the toxicological profiles are health professionals at the Federal, State, and local levels; interested private sector organizations and groups; and members of the public. We plan to revise these documents in response to public comments and as additional data become available. Therefore, we encourage comments that will make the toxicological profile series of the greatest use.

Electronic comments may be submitted via: www.regulations.gov.
Follow the on-line instructions for submitting comments.

Written comments may also be sent to:

Agency for Toxic Substances and Disease Registry
Division of Toxicology and Human Health Sciences
Environmental Toxicology Branch

Regular Mailing Address:
1600 Clifton Road, N.E.
Mail Stop F-57
Atlanta, Georgia 30329-4027

Physical Mailing Address:
4770 Buford Highway
Building 102, 1st floor, MS F-57
Chamblee, Georgia 30341

The toxicological profiles are developed under the Comprehensive Environmental Response, Compensation, and Liability Act of 1980, as amended (CERCLA or Superfund). CERCLA section 104(i)(1) directs the Administrator of ATSDR to "…effectuate and implement the health related authorities" of the statute. This includes the preparation of toxicological profiles for hazardous substances most commonly found at facilities on the CERCLA National Priorities List and that pose the most significant potential threat to human health, as determined by ATSDR and the EPA. Section 104(i)(3) of CERCLA, as amended, directs the Administrator of ATSDR to prepare a toxicological profile for each substance on the list. In addition, ATSDR has the authority to prepare toxicological profiles for substances not found at sites on the National Priorities List, in an effort to "…establish and maintain inventory of literature, research, and studies on the health effects of toxic substances" under CERCLA Section 104(i)(1)(B), to respond to requests for consultation under section 104(i)(4), and as otherwise necessary to support the site-specific response actions conducted by ATSDR.

This profile reflects ATSDR's assessment of all relevant toxicologic testing and information that has been peer-reviewed. Staffs of the Centers for Disease Control and Prevention and other Federal scientists have also reviewed the profile. In addition, this profile has been peer-reviewed by a nongovernmental panel and is being made available for public review. Final responsibility for the contents and views expressed in this toxicological profile resides with ATSDR.

Patrick N. Breysse, Ph.D., CIH
Director, National Center for Environmental Health and
Agency for Toxic Substances and Disease Registry
Centers for Disease Control and Prevention

QUICK REFERENCE FOR HEALTH CARE PROVIDERS

Toxicological Profiles are a unique compilation of toxicological information on a given hazardous substance. Each profile reflects a comprehensive and extensive evaluation, summary, and interpretation of available toxicologic and epidemiologic information on a substance. Health care providers treating patients potentially exposed to hazardous substances may find the following information helpful for fast answers to often-asked questions.

Primary Chapters/Sections of Interest

Chapter 1: Public Health Statement: The Public Health Statement can be a useful tool for educating patients about possible exposure to a hazardous substance. It explains a substance's relevant toxicologic properties in a nontechnical, question-and-answer format, and it includes a review of the general health effects observed following exposure.

Chapter 2: Relevance to Public Health: The Relevance to Public Health Section evaluates, interprets, and assesses the significance of toxicity data to human health.

Chapter 3: Health Effects: Specific health effects of a given hazardous compound are reported by type of health effect (e.g.,death, systemic, immunologic, reproductive), by route of exposure, and by length of exposure (acute, intermediate, and chronic). In addition, both human and animal studies are reported in this section.
NOTE: Not all health effects reported in this section are necessarily observed in the clinical setting. Please refer to the Public Health Statement to identify general health effects observed following exposure.

Pediatrics: Four new sections have been added to each Toxicological Profile to address child health issues:

Chapter 1	**How Can (Chemical X) Affect Children?**
Chapter 1	**How Can Families Reduce the Risk of Exposure to (Chemical X)?**
Section 3.7	**Children's Susceptibility**
Section 6.6	**Exposures of Children**

Other Sections of Interest:

Section 3.8	**Biomarkers of Exposure and Effect**
Section 3.11	**Methods for Reducing Toxic Effects**

ATSDR Information Center

Phone: 1-800-CDC-INFO (800-232-4636) or 1-888-232-6348 (TTY)
Internet: http://www.atsdr.cdc.gov

The following additional materials are available online:

Case Studies in Environmental Medicine are self-instructional publications designed to increase primary health care providers' knowledge of a hazardous substance in the environment and to aid in the evaluation of potentially exposed patients (see https://www.atsdr.cdc.gov/csem/csem.html).

Managing Hazardous Materials Incidents is a three-volume set of recommendations for on-scene (prehospital) and hospital medical management of patients exposed during a hazardous materials incident (see https://www.atsdr.cdc.gov/MHMI/index.asp). Volumes I and II are planning guides to assist first responders and hospital emergency department personnel in planning for incidents that involve hazardous materials. Volume III—*Medical Management Guidelines for Acute Chemical Exposures*—is a guide for health care professionals treating patients exposed to hazardous materials.

Fact Sheets (ToxFAQs™) provide answers to frequently asked questions about toxic substances (see https://www.atsdr.cdc.gov/toxfaqs/Index.asp).

Other Agencies and Organizations

The National Center for Environmental Health (NCEH) focuses on preventing or controlling disease, injury, and disability related to the interactions between people and their environment outside the workplace. Contact: NCEH, Mailstop F-29, 4770 Buford Highway, NE, Atlanta, GA 30341-3724 • Phone: 770-488-7000 • FAX: 770-488-7015 • Web Page: https://www.cdc.gov/nceh/.

The National Institute for Occupational Safety and Health (NIOSH) conducts research on occupational diseases and injuries, responds to requests for assistance by investigating problems of health and safety in the workplace, recommends standards to the Occupational Safety and Health Administration (OSHA) and the Mine Safety and Health Administration (MSHA), and trains professionals in occupational safety and health. Contact: NIOSH, 395 E Street, S.W., Suite 9200, Patriots Plaza Building, Washington, DC 20201 • Phone: 202-245-0625 or 1-800-CDC-INFO (800-232-4636) • Web Page: https://www.cdc.gov/niosh/.

The National Institute of Environmental Health Sciences (NIEHS) is the principal federal agency for biomedical research on the effects of chemical, physical, and biologic environmental agents on human health and well-being. Contact: NIEHS, PO Box 12233, 104 T.W. Alexander Drive, Research Triangle Park, NC 27709 • Phone: 919-541-3212 • Web Page: https://www.niehs.nih.gov/.

Clinical Resources (Publicly Available Information)

The Association of Occupational and Environmental Clinics (AOEC) has developed a network of clinics in the United States to provide expertise in occupational and environmental issues. Contact: AOEC, 1010 Vermont Avenue, NW, #513, Washington, DC 20005 • Phone: 202-347-4976 • FAX: 202-347-4950 • e-mail: AOEC@AOEC.ORG • Web Page: http://www.aoec.org/.

The American College of Occupational and Environmental Medicine (ACOEM) is an association of physicians and other health care providers specializing in the field of occupational and environmental medicine. Contact: ACOEM, 25 Northwest Point Boulevard, Suite 700, Elk Grove Village, IL 60007-1030 • Phone: 847-818-1800 • FAX: 847-818-9266 • Web Page: http://www.acoem.org/.

The American College of Medical Toxicology (ACMT) is a nonprofit association of physicians with recognized expertise in medical toxicology. Contact: ACMT, 10645 North Tatum Boulevard,

Suite 200-111, Phoenix AZ 85028 • Phone: 844-226-8333 • FAX: 844-226-8333 • Web Page: http://www.acmt.net.

The Pediatric Environmental Health Specialty Units (PEHSUs) is an interconnected system of specialists who respond to questions from public health professionals, clinicians, policy makers, and the public about the impact of environmental factors on the health of children and reproductive-aged adults. Contact information for regional centers can be found at http://pehsu.net/findhelp.html.

The American Association of Poison Control Centers (AAPCC) provide support on the prevention and treatment of poison exposures. Contact: AAPCC, 515 King Street, Suite 510, Alexandria VA 22314 • Phone: 701-894-1858 • Poison Help Line: 1-800-222-1222 • Web Page: http://www.aapcc.org/.

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THE PROFILE HAS UNDERGONE THE FOLLOWING ATSDR INTERNAL REVIEWS:

1. Health Effects Review. The Health Effects Review Committee examines the health effects chapter of each profile for consistency and accuracy in interpreting health effects and classifying end points.

2. Minimal Risk Level Review. The Minimal Risk Level Workgroup considers issues relevant to substance-specific Minimal Risk Levels (MRLs), reviews the health effects database of each profile, and makes recommendations for derivation of MRLs.

3. Data Needs Review. The Environmental Toxicology Branch reviews data needs sections to assure consistency across profiles and adherence to instructions in the Guidance.

4. Green Border Review. Green Border review assures the consistency with ATSDR policy.

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PEER REVIEW

A peer review panel was assembled for 2,4-D. The panel consisted of the following members:

1. Dr. Lucio G. Costa, Department of Environmental and Occupational Health Sciences, University of Washington, Seattle, Washington;

2. Dr. Aaron E. Blair, Division of Cancer Epidemiology and Genetics, National Cancer Institute, Rockville, Maryland; and

3. Dr. Nancy K. Wilson, Wilson Associates, Chapel Hill, North Carolina.

These experts collectively have knowledge of 2,4-D's physical and chemical properties, toxicokinetics, key health end points, mechanisms of action, human and animal exposure, and quantification of risk to humans. All reviewers were selected in conformity with the conditions for peer review specified in Section 104(I)(13) of the Comprehensive Environmental Response, Compensation, and Liability Act, as amended.

Scientists from the Agency for Toxic Substances and Disease Registry (ATSDR) have reviewed the peer reviewers' comments and determined which comments will be included in the profile. A listing of the peer reviewers' comments not incorporated in the profile, with a brief explanation of the rationale for their exclusion, exists as part of the administrative record for this compound.

The citation of the peer review panel should not be understood to imply its approval of the profile's final content. The responsibility for the content of this profile lies with the ATSDR.

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1. PUBLIC HEALTH STATEMENT FOR 2,4-D

This Public Health Statement summarizes the Agency for Toxic Substances and Disease Registry's (ATSDR) findings on 2,4-dichlorophenoxyacetic acid (2,4-D), including chemical characteristics, exposure risks, possible health effects from exposure, and ways to limit exposure.

The U.S. Environmental Protection Agency (EPA) identifies the most serious hazardous waste sites in the nation. These sites make up the National Priorities List (NPL) and are sites targeted for long-term federal clean-up activities. The EPA has found 2,4-D in at least 46 of the 1,832 current or former NPL sites. The total number of NPL sites evaluated for 2,4-D is not known. But the possibility remains that as more sites are evaluated, the sites where 2,4-D is found may increase. This information is important because these future sites may be sources of exposure, and exposure to 2,4-D may be harmful.

If you are exposed to 2,4-D, many factors determine whether you'll be harmed. These include how much you are exposed to (dose), how long you are exposed (duration), how often you are exposed (frequency), and how you are exposed (route of exposure). You must also consider the other chemicals you are exposed to and your age, sex, diet, family traits, lifestyle, and state of health.

WHAT IS 2,4-D?

2,4-Dichlorophenoxyacetic acid (2,4-D) does not occur naturally in the environment. 2,4-D is the active ingredient in many products used in the United States and throughout the world as an herbicide to kill weeds on land and in the water. There are nine forms of 2,4-D that can be used as an herbicide and it is typically sold as a powder or in a liquid form.

WHAT HAPPENS TO 2,4-D WHEN IT ENTERS THE ENVIRONMENT?

2,4-D can be released into the air when it is being applied to weeds and can be released when it is being made. 2,4-D in the air can be broken down by other chemicals or can settle to the ground. It takes about 19 hours to break down half of the 2,4-D in the air. 2,4-D is not persistent in most soils. Its half-life in soils is about 6 days under aerobic conditions (environments where oxygen is present) but longer under anaerobic (environments where there is limited oxygen) conditions. Although some of the 2,4-D in soil can go through the soil and enter the groundwater, it is rarely detected in groundwater. 2,4-D can enter rivers, lakes, and ponds when 2,4-D is sprayed on nearby plants, from runoff and soil erosion, or when it

is used on water plants. It breaks down more slowly in water than it does in the air or in soil. It takes about 15 days to break down half of the 2,4-D in water under aerobic conditions and about 41–333 days under anaerobic conditions. 2,4-D is not likely to concentrate in fish.

HOW MIGHT I BE EXPOSED TO 2,4-D?

Many herbicidal products contain 2,4-D. You may be exposed to 2,4-D when applying these products if you breathe it in or get it on your skin, especially if you eat afterwards without washing your hands or smoke during applications. You may also be exposed to 2,4-D while walking or playing on very recently treated lawns, gardens, golf courses, parks, or other grassy areas. People and pets may transport 2,4-D into homes by walking across recently treated lawns. You may also be exposed to 2,4-D in soils of treated lawns. Swimming in areas that use 2,4-D to control weeds is another way that you may come in contact with it. When workers make 2,4-D or apply it to weeds, they may have higher exposures. You are unlikely to be exposed to high levels of 2,4-D in food, water, or soil. It was detected at low levels (levels near the detection limits of the measurement) in roughly 20% of the food samples tested by the FDA and when it is found in drinking waters, it is usually well below the acceptable levels that EPA considers safe.

HOW CAN 2,4-D ENTER AND LEAVE MY BODY?

2,4-D can enter your body when you drink water or eat food containing 2,4-D. Almost all of the 2,4-D can be taken up (absorbed) from the gastrointestinal tract and enter the bloodstream within a few hours. A small amount of 2,4-D can enter your body through your skin. It has not been determined how much can enter through your lungs. The 2,4-D that is absorbed will enter the blood and move throughout your body. 2,4-D is found in most organs in your body. Your body does not break down or change 2,4-D. It may leave your body in the urine around 24 hours after a single initial exposure and if exposure is no longer occurring. 2,4-D does not accumulate in the body.

Additional information regarding how 2,4-D can enter and leave the body can be found in Section 3.4.

HOW CAN 2,4-D AFFECT MY HEALTH?

If you follow the manufacturer’s instructions, you are not likely to experience the harmful effects of 2,4-D. It does not appear that contact with small amounts of 2,4-D will cause harmful effects in humans

based on currently available scientific evidence. Studies in laboratory animals have found a number of effects:

- Decreases in the amount of breast milk mothers produced
- Alterations in blood
- Liver effects
- Kidney effects
- Alterations in thyroid hormone levels

In general, effects were seen when the animals were given 2,4-D doses that were much higher than people would come in contact with in the environment.

Harmful effects have been seen in people who purposely or accidentally swallowed large amounts of 2,4-D; much larger amounts than found in the environment. These serious effects, which include fast breathing and heart rate, vomiting, confusion, coma, and paralysis, are not likely to occur at the levels of 2,4-D that are generally found in the environment. It should be mentioned that commercial herbicides that contain 2,4-D may have other substances in them. Some of these effects may be due to exposure to these other substances.

A few studies of farmers or professional applicators of herbicides containing 2,4-D have found that use or exposure to 2,4-D was linked with harmful health effects, particularly some cancers of the lymph system (i.e., bone marrow, thymus gland, lymph nodes, tonsils, spleen). These studies were in workers who are exposed to higher amounts of 2,4-D than most people. Some studies found increases in Non-Hodgkin's lymphoma (NHL), which is a type of cancer. However, most human studies did not find strong proof that exposure to just 2,4-D increased the risk of developing NHL; there was not strong proof for links between 2,4-D exposure and other types of cancer. Long-term oral exposure of rats, mice, or dogs to 2,4-D did not produce cancer in any of these animal species.

The EPA has determined that 2,4-D is not classifiable as to human carcinogenicity (Group D). This means that there was not adequate data either to support or refute human carcinogenicity. The International Agency for Research on Cancer (IARC) recently classified 2,4-D as possibly carcinogenic to humans (Group 2B) based on "inadequate evidence" in humans and "limited evidence" in experimental animals.

Additional information regarding 2,4-D and health effects can be found in Section 3.2.

HOW CAN 2,4-D AFFECT CHILDREN?

This section discusses potential health effects of 2,4-D exposure in humans from when they're first conceived to 18 years of age.

Studies of children living in farming areas did not find meaningful links between exposure to 2,4-D and harmful health effects. These studies included looking into birth weight and how often birth defects and cancer occurred in children exposed to 2,4-D. A study did find that the male children of mothers exposed to 2,4-D during pregnancy had an increased risk of hay fever or allergies when they were aged 12 years or older. Because all of these studies suffer limitations that may have influenced the results into finding positive or negative links, no firm conclusions can be drawn from them.

Studies in animals have shown that 2,4-D can be transferred to the fetus across the placenta and to newborn animals through maternal milk. Although this has not been directly shown in humans, it seems sensible to believe that it could happen.

Some studies in animals have shown that exposure to 2,4-D during and after pregnancy can reduce the weight of the fetuses and young animals during the first weeks of life. It also can cause minor tissue abnormalities without meaningful lasting effects. In one study, pups from rats exposed to 2,4-D during and after pregnancy showed changes in some behaviors such as spontaneous movement and grooming. This occurred when the rats were given 2,4-D doses that were much higher than people would come in contact with in the environment. 2,4-D did not cause birth defects in animals.

Additional information regarding 2,4-D and health effects in children can be found in Section 3.7.

HOW CAN FAMILIES REDUCE THE RISK OF EXPOSURE TO 2,4-D?

If your doctor finds that you have been exposed to significant amounts of 2,4-D, ask whether your children might also be exposed. Your doctor might need to ask your state health department to investigate. You may also contact the state or local health department with health concerns.

2,4-D and its different chemical forms are listed as an ingredient in about 600 farm and household products. We recommend that you follow the directions when using 2,4-D products. It is especially important to wait until the sprayed area is dry and do not walk barefoot in the area. Wear protective eye

gear and gloves when using 2,4-D-containing products in order to reduce exposure. Getting 2,4-D on the skin is the main way that you can be exposed. Wear protective clothing to lessen skin contact. Do not stand in spray drift when 2,4-D containing herbicides are applied. Do not smoke while applying or in areas recently treated with 2,4-D. Amounts remaining on the skin after contact can easily be transferred to the mouth, other body parts, or other surfaces. This could result in "second-hand" exposures, which may be especially important for children. Washing after using 2,4-D products will lessen exposure to it and reduce unintentional hand to mouth ingestion. 2,4-D has been detected in the urine of dogs that have played on treated lawns. Prevent children and pets from playing on lawns treated recently with 2,4-D.

ARE THERE MEDICAL TESTS TO DETERMINE WHETHER I HAVE BEEN EXPOSED TO 2,4-D?

There are tests to measure 2,4-D in blood, urine, and tissues of the body. Urine is easy to collect, so measuring 2,4-D in urine is the favored test to use. Finding 2,4-D in your body does not always mean that you will have harmful health effects. Most of the 2,4-D in the body does not breakdown. It does not build up in the body. 2,4-D leaves the body in the urine around 24 hours after a single exposure. Get tests for 2,4-D done quickly after exposure. Doctor's offices do not normally do these types of tests. Specialized laboratories will test the samples.

More information about ways to measure 2,4-D in the body can be found in Chapter 7.

WHAT RECOMMENDATIONS HAS THE FEDERAL GOVERNMENT MADE TO PROTECT HUMAN HEALTH?

The federal government develops regulations and recommendations to protect public health. Regulations can be enforced by law. Federal agencies that develop regulations for toxic substances include the Environmental Protection Agency (EPA), the Occupational Safety and Health Administration (OSHA), and the Food and Drug Administration (FDA). Recommendations provide valuable guidelines to protect public health but are not enforceable by law. Federal organizations that develop recommendations for toxic substances include the Agency for Toxic Substances and Disease Registry (ATSDR) and the National Institute for Occupational Safety and Health (NIOSH).

Regulations and recommendations can be expressed as "not-to-exceed" levels; that is, levels of a toxic substance in air, water, soil, or food that do not exceed a critical value usually based on levels that affect animals; levels are then adjusted to help protect humans. Sometimes these not-to-exceed levels differ

among federal organizations. Different organizations use different exposure times (e.g., an 8-hour workday or a 24-hour day), different animal studies, or emphasize some factors over others, depending on their mission.

Recommendations and regulations are also updated periodically as more information becomes available. For the most current information, check with the federal agency or organization that issued the regulation or recommendation.

EPA has made recommendations on the acceptable levels of 2,4-D in drinking water that would be safe for a child weighing 10 kilograms (10 kg or 22 pounds). The level that would be safe for a 1-day exposure is 1 milligram per liter (1 mg/L). The level that would be safe for a 10-day exposure is 0.3 mg/L.

OSHA established a legal limit of 10 milligrams per cubic meter (10 mg/m^3) as an average for 2,4-D in workplace air during an 8-hour workday.

NIOSH recommends an exposure limit of 10 mg/m^3 for 2,4-D in workplace air during a 10-hour workday. NIOSH also says that an air level of 100 mg 2,4-D/m^3 is an immediate danger to life or health.

FDA set an allowable limit of no more than 0.07 mg 2,4-D/L in bottled drinking water.

WHERE CAN I GET MORE INFORMATION?

If you have any questions or concerns, please contact your community or state health or environmental quality department, or contact ATSDR at the address and phone number below. You may also contact your doctor if experiencing adverse health effects or for medical concerns or questions. ATSDR can also provide publicly available information regarding medical specialists with expertise and experience recognizing, evaluating, treating, and managing patients exposed to hazardous substances.

- Call the toll-free information and technical assistance number at 1-800-CDCINFO (1-800-232-4636) or

- Write to:
  Agency for Toxic Substances and Disease Registry
  Division of Toxicology and Human Health Sciences
  1600 Clifton Road NE
  Mailstop F-57
  Atlanta, GA 30329-4027

Toxicological profiles and other information are available on ATSDR's web site: http://www.atsdr.cdc.gov.

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2. RELEVANCE TO PUBLIC HEALTH

2.1 BACKGROUND AND ENVIRONMENTAL EXPOSURES TO 2,4-D IN THE UNITED STATES

2,4-D is a free acid pesticide widely used in the United States (see Table 4-1). While the free acid is itself used as an herbicide, there are nine forms of 2,4-D registered as active ingredients in end use products. These include salts, amines, and esters of 2,4-D. Derivatives include the sodium salt, diethanolamine salt, dimethyl amine salt, isopropylamine salt, triisopropanolamine salt, butoxyethyl ester, ethylhexyl ester, and isopropyl ester. Almost 90–95% of total global use is accounted for by dimethyl amine salt and ethylhexyl ester. 2,4-D and its different chemical forms are listed as an ingredient, either as the singular active ingredient or in conjunction with other ingredients, in about 600 agricultural and residential products. 2,4-D is one of the most widely used agricultural herbicides in the United States with approximately 39 million pounds applied to crops in 2013, with pasture and hay fields and wheat, soybean, and corn crops receiving the greatest applications. It is also applied to residential or commercial turf for the elimination of a wide variety of broadleaf weeds without causing harm to the grass.

The dominant process affecting the overall environmental fate, transport, and bioaccumulation of 2,4-D is degradation by microbiological activity. 2,4-D has been shown to undergo degradation in pure cultures by particular species of microorganisms. The two main pathways of degradation are via a hydroxyphenoxy acetic acid intermediate or by the corresponding phenol. The half-life of 2,4-D was about 6 days when it was applied to a mineral soil maintained under aerobic conditions. 2,4-D is likely to migrate through the soil and into groundwater since it has high mobility in soils under varying conditions. 2,4-D is not expected to volatilize from water or soil surfaces since most forms of 2,4-D are supplied as amine salts, which do not volatilize, and the ester forms are rapidly transformed to the corresponding acid, which will exist as an anion under environmental conditions. Data suggest that bioconcentration of 2,4-D does not occur to a significant extent in aquatic organisms.

The general population may be exposed to 2,4-D during and after its use in residential and recreational areas. 2,4-D applications often occur to residential lawns, golf courses, parks, cemeteries, and other grassy areas. Since 2,4-D is also used on aquatic weeds, swimmers may also be exposed. 2,4-D can unintentionally be transported into residences if clothing or shoes containing this substance are worn indoors or if pets track in 2,4-D from recently treated lawns. The general population can be exposed to 2,4-D by ingesting food or water contaminated with it or through dermal contact with it when used in residential settings (lawn applications). Populations living within or very near areas of heavy agricultural

2,4-D use have an increased risk of exposure to relatively larger amounts of 2,4-D through dermal contact with contaminated plants, soils, or surface waters or by inhalation of the mist formed from the applied herbicide. Those likely to receive the highest exposures are those who are involved in the production, formulation, handling, and application of 2,4-D. Dermal contact appears to be the major route of exposure for workers, although inhalation exposure and accidental ingestion via hand-to-mouth activity is possible.

Children are expected to be exposed to 2,4-D by the same routes that affect adults. Small children are more likely to come into contact with 2,4-D residues that may be present in soil and dust, due to increased hand-to-mouth activity and playing habits. However, dermal contact with house dust contaminated with small residues of 2,4-D is the most likely route of exposure for children. Treated play areas (lawns) and pets that may have come in contact with 2,4-D on treated lawns is another possible source of exposure. No human data were located regarding 2,4-D in breast milk; therefore, an adequate determination of the importance of this route of child exposure has not been made.

2.2 SUMMARY OF HEALTH EFFECTS

Information regarding health effects in humans following exposure to 2,4-D comes from case reports of accidental or intentional ingestion of herbicide formulations containing 2,4-D, accidental skin contact with those products by farmers and professional residential applicators and homeowners (see Section 3.2.3, Dermal Exposure, for multiple references), and occupational exposure during manufacture. Effects that have been reported following oral or dermal exposure to high amounts of 2,4-D include tachypnea, tachycardia, vomiting, leukocytosis, liver and kidney congestion in fatal cases, metabolic acidosis, and neurological effects characterized by sensory and motor abnormalities. In two reports of dermal exposure, signs and symptoms of peripheral neuropathy persisted for a long time. Some of these studies estimated exposure levels and/or measured levels of 2,4-D in the body. A report estimated an ingested dose of approximately 80 mg/kg in a fatal case. In another fatal case, the investigators estimated that the subject had ingested at least 25–35 g of 2,4-D (357–500 mg/kg for a 70 kg body weight). However, there is a report of two individuals who survived after ingesting approximately 40 and 140 g of 2,4-D (571 and 2,000 mg/kg) in herbicide products. It should be kept in mind that these numbers are the result of the combined action of 2,4-D and other substances in the commercial formulations. In addition, whether or not deaths occurred may be related to the time elapsed between poisoning and beginning of emergency medical treatment.

Numerous epidemiological studies, mostly case-control and cohort studies, have examined potential associations between exposure to 2,4-D and multiple health outcomes including respiratory effects, endocrine effects, ocular effects, body weight effects, immunological effects, neurological effects, reproductive effects, developmental effects, various cancers (see Section 3.2.3.7, Cancer, for multiple references), and death.

While some of the human studies reported significant associations between use/exposure to 2,4-D and adverse health outcomes, some did not. It should be kept in mind also that pesticide applicators and farm workers are likely to be exposed to multiple chemicals, and even if analyses can be conducted for exposures to individual chemicals, a significant association between use/exposure and increased prevalence of an adverse health outcome does not necessarily imply causality, although it suggests that exposure to the chemical plays a role in the health outcome assessed and that biological plausibility exists. In general, issues that limited the interpretation of both positive and negative associations reported included lack of relationship with frequency of use of 2,4-D or the amounts of 2,4-D used, duration of exposure, or too few cases reported for a meaningful interpretation.

Among the various types of cancers examined (lymphatic system cancers, gastrointestinal cancer, breast cancer, cancers of the nervous system, prostate cancer, and others), lymphatic system cancers, in particular non-Hodgkin's lymphoma (NHL), has received the most attention and has been the subject of several reviews. Some case-control studies reported that exposure to 2,4-D increased the risk of NHL, but others did not. The latter included cases of agriculture exposure, residential use of 2,4-D, exposure during manufacture, or in children from parents participating in the Agricultural Health Study (AHS). The AHS is a prospective cohort study of nearly 90,000 private pesticide applicators (mostly farmers), their spouses, and commercial pesticide applicators in Iowa and North Carolina. The AHS is funded by the National Cancer Institute and the National Institute of Environmental Health Sciences in collaboration with the EPA and NIOSH. No significant differences were reported in a few studies that assessed combinations of 2,4-D and other phenoxy acids such as 2,4,5-T or 2,4-dichlorophenoxypropionic acid (2,4-DP) and 2,4-dichlorophenoxybutyric acid (2,4-DB). Studies that examined cause-specific mortality among employees engaged in the manufacture, formulation, or packaging of 2,4-D and related salts did not find patterns suggestive of a causal association between exposure to 2,4-D and any particular cause of death, including NHL. Overall, 2,4-D has exhibited low toxicity in studies of humans environmentally or occupationally exposed to this chemical.

The database in animals is extensive and consists mostly of studies by the oral route of exposure. In the only inhalation study available, intermittent nose-only exposure of rats to 2,4-D dusts for 28 days resulted in relatively low systemic toxicity; however, the lowest concentration tested, 50 mg/m^3, induced histological alterations in the larynx (portal-of entry effect). Oral studies in animals have reported a wide range of effects in acute-, intermediate-, and chronic-duration studies. Acute-duration studies have reported LD_{50} values ranging from 100 mg/kg in dogs to 1,000 mg/kg in guinea pigs. Dogs appear to be more sensitive than rats and mice. This appears to be due to dogs having a significantly lower capacity to eliminate 2,4-D via the kidneys than other species, including humans. Systemic effects reported in repeated exposure oral studies include hematological alterations in rats (decreased hemoglobin, platelets, and erythrocyte counts); hepatic effects in rats (histological alterations) and dogs (perivascular inflammation); renal effects in rats, mice, and dogs; alterations in thyroid hormone levels in rats; ocular effects in rats; and alterations in body weight gain in most species tested. Some apparent inconsistent results between studies, particularly regarding hepatic, renal, and thyroid effects, make it difficult to make generalizations and define reliable no-observed-adverse-effect levels (NOAELs) and lowest-observed-adverse-effect levels (LOAELs). For example, a 13-week study reported a LOAEL of approximately 7.1 mg 2,4-D/kg/day for histological lesions in the kidneys of rats. However, other 13-week or shorter duration studies in rats reported LOAELs for histological alterations in the kidneys only at doses ≥20 mg 2,4-D/kg/day. In another study, female rats exposed to ≥15 mg 2,4-D/kg/day for 27 weeks had significantly increased serum thyroxine (T4), but no increase was evident after 52 weeks of exposure and no alterations were seen in males exposed to up to 45 mg 2,4-D/kg/day at either time point. In addition, the toxicological significance of the results from some studies is not clear, as is the case, for example, for alterations in the kidneys from rats and mice characterized as increased homogeneity of the cytoplasm and decreased vacuolization of cells in the renal cortex. Studies in animals suggest that the respiratory, gastrointestinal, and cardiovascular systems are not sensitive targets for 2,4-D toxicity.

Results from *in vivo* and *in vitro* studies showed no evidence that 2,4-D is an endocrine disruptor substance. The EPA recently completed a weight-of-evidence analysis of the potential interaction of 2,4-D with the androgen, estrogen, and thyroid signaling pathways and concluded that there is no convincing evidence of interaction with any of the three pathways.

Exposure to 2,4-D did not affect the gross or microscopic morphology of lymphoreticular organs and tissues of animals as shown in multiple studies. Oral exposure of rats to 2,4-D did not affect immunocompetence, assessed by the sheep red blood cell (SRBC) antibody plaque forming cell assay. 2,4-D was a respiratory allergen in mice following dermal sensitization and challenge with the chemical

intratracheally. This information is insufficient to draw conclusions regarding 2,4-D and the immune system.

In general, exposure to 2,4-D did not induce gross or microscopic alterations in tissues of the nervous system of animals, but a relatively high single dose of 150 mg 2,4-D/kg altered the blood brain barrier in rats leading to vascular damage in the central nervous system. Exposure to 2,4-D induced neurobehavioral alterations in some studies. Worth noting is a relatively low LOAEL of 15 mg 2,4-D/kg/day (the lowest dose tested) for altered maternal behavior in rats dosed on postpartum days 1–6. Specifically, the effects consisted of increased latency of retrieval of pups, increased latency of crouching, decreased percent dams licking the pups, decreased percent dams licking the anogenital region of the pups, increased percent of dams leaving the nest, and increased time spent out of the nest. These behaviors were associated with a decrease in serotonin and an increase in dopamine in the arcuate nucleus of the brain. Single high doses of 250 mg 2,4-D/kg altered gait and motor activity in rats, whereas repeated doses of ≥20 mg 2,4-D/kg/day increased grip strength. The available data suggest that 2,4-D is not a neurotoxic substance at environmentally relevant doses (in the low µg/kg body weight/day range). However, it is unknown whether neurobehavioral alterations could occur as a result of chronic-duration exposure to low doses of 2,4-D. Available chronic-duration studies did not conduct neurobehavioral tests.

Exposure of male and female animals to 2,4-D through the diet did not affect the morphology of reproductive organs, nor did it affect mating and fertility indices or sperm parameters. However, histological alterations in Sertoli and Leydig cells and reduced sperm count and motility were reported in rats administered ≥50 mg 2,4-D/kg/day by gavage for 30 days. There is no explanation for this apparent discrepancy in results regarding sperm parameters other than the different modes of administering 2,4-D to the animals (i.e., diet versus gavage). Studies with exposure routes relevant to general population exposures suggest that 2,4-D is not a reproductive toxicant.

Perinatal exposure to 2,4-D has resulted in developmental effects, mostly reduced fetal or offspring weight and minor soft-tissue and skeletal anomalies, in some studies, but it did not induce teratogenicity. In many cases, reduced fetal weight was accompanied by reduced maternal weight gain during pregnancy or some other maternal effect. A low LOAEL of 2.5 mg 2,4-D/kg/day was reported for reduced body weight in 10-day-old rat pups from dams exposed on postpartum days 1–16. The effect was attributed to inhibition of the suckling-induced hormone release milk transfer to the litter by an action of 2,4-D at the level of the central nervous system. Other studies have reported reduced offspring weight but at higher

maternal exposure levels. Other developmental effects reported include neurobehavioral alteration in rat pups and delayed vaginal opening at maternal doses of 70 mg 2,4-D/kg/day and histological alterations in rat pup liver and bone at maternal doses of 126 mg 2,4-D kg/day. 2,4-D did not induce developmental effects in hamsters following maternal exposure to ≤100 mg 2,4-D/kg/day or in rabbits following maternal exposure to ≤90 mg 2,4-D/kg/day. With the exception of the relatively low LOAEL of 2.5 mg/kg/day for reduced offspring weight, 2,4-D does not appear to be a strong developmental toxicant.

2,4-D was not carcinogenic in oral bioassays in rats, mice, and dogs. The EPA has assigned 2,4-D to carcinogenicity Group D, "not classifiable as to human carcinogenicity". The International Agency for Research on Cancer (IARC) recently classified 2,4-D as possibly carcinogenic to humans (Group 2B) based on inadequate evidence in humans and limited evidence in experimental animals. In discussing potential mechanisms by which 2,4-D could induce cancer, IARC noted that the evidence that 2,4-D induces oxidative stress that can operate in humans is strong, the evidence that 2,4-D is genotoxic is weak, the evidence that 2,4-D causes immunosuppression is moderate, the evidence that 2,4-D modulates receptor activity is weak, and the evidence that 2,4-D alters cell proliferation or death is weak. Recently, Canada's Pest Management Regulatory Agency (PMRA) concluded that 2,4-D cannot be classified as a human carcinogen based on the inconsistent epidemiological associations, the recognition that that there are many other factors that may contribute to the etiology of the reported cancer cases, information from the PMRA's incident report database, and the fact that the weight of evidence from animal studies designed to show causality did not support a carcinogenic effect.

2.3 MINIMAL RISK LEVELS (MRLs)

Estimates of exposure levels posing minimal risk to humans (MRLs) have been established for 2,4-D. An MRL is defined as an estimate of daily human exposure to a substance that is likely to be without an appreciable risk of adverse effects (noncarcinogenic) over a specified duration of exposure. MRLs are derived when reliable and sufficient data exist to identify the target organ(s) of effect or the most sensitive health effect(s) for a specific duration within a given route of exposure. MRLs are based on noncancerous health effects only and do not consider carcinogenic effects. MRLs can be derived for acute, intermediate, and chronic duration exposures for inhalation and oral routes. Appropriate methodology does not exist to develop MRLs for dermal exposure.

Although methods have been established to derive these levels (Barnes and Dourson 1988; EPA 1990), uncertainties are associated with these techniques. Furthermore, ATSDR acknowledges additional

uncertainties inherent in the application of the procedures to derive less than lifetime MRLs. As an example, acute inhalation MRLs may not be protective for health effects that are delayed in development or are acquired following repeated acute insults, such as hypersensitivity reactions, asthma, or chronic bronchitis. As these kinds of health effects data become available and methods to assess levels of significant human exposure improve, these MRLs will be revised.

Inhalation MRLs

No inhalation MRLs were derived for 2,4-D. Only one inhalation study was available for review. In that study, male and female rats were exposed nose-only 6 hours/day, 5 days/week for 28 days to 2,4-D dusts in target concentrations of 0, 50, 100, 300, and 1,000 mg/m^3 (EPA 2008). After termination of exposure, controls and rats from the highest exposure concentration group were kept for a 4-week recovery period to assess reversibility of the effects. A significant reduction in reticulocytes occurred in males and females at ≥300 mg/m^3 2,4-D and a significant increase in serum alkaline phosphatase was reported in females at ≥300 mg/m^3 2,4-D. No significant histological alterations were reported in the tissues and organs examined. The most salient effect was the occurrence of squamous/squamoid epithelial metaplasia with hyperkeratosis in the larynx of all exposed groups, with increasing severity as the exposure concentration increased. The lesions persisted during the recovery period, but with reduced severity. Therefore, the exposure concentration of 50 mg/m^3 2,4-D represents the study LOAEL, a portal-of-entry LOAEL. Although this is a well-conducted study that examined a comprehensive number of end points, the database is insufficient for MRL derivation. It would be important to determine a NOAEL for the portal-of-entry effects.

Oral MRLs

An acute-duration oral MRL for 2,4-D was not derived. However, it is recommended that the intermediate-duration oral of 0.009 mg 2,4-D/kg/day be adopted also as acute-duration oral MRL for 2,4-D based on the information discussed below.

No adequate acute human data were located. Information regarding health effects in humans following acute-duration exposure to 2,4-D is limited to case reports of intentional or accidental ingestion of herbicide formulations containing 2,4-D. Effects that have been reported following oral exposure to high amounts of 2,4-D include tachypnea, tachycardia, vomiting, leukocytosis, liver and kidney congestion in fatal cases, metabolic acidosis, and death (Dudley and Thapar 1972; Durakovic et al. 1992; Keller et al.

1994; Nielsen et al. 1965; Smith and Lewis 1987). While some of these studies provided estimates of amounts of 2,4-D ingested, as stated earlier, the reported effects represent the result of exposure to a chemical mixture consisting of 2.4-D and other substances present in the commercial formulations (i.e., solvents, other herbicides), which is the exposure that most humans experience. Yet, the common exposure reported across studies was to 2,4-D. Two studies with volunteers in which the subjects were administered a single gelatin capsule containing a dose of 5 mg/kg 2,4-D reported no ill effects among the volunteers during the 1-week monitoring period that followed dosing (Kohli et al. 1974; Sauerhoff et al. 1977). Without specifying, Sauerhoff et al. (1977) stated that no untoward effects were associated with ingestion of 2,4-D. Kohli et al. (1974) monitored blood pressure, heart rate, hemoglobin content, and total and differential white cell counts and stated that no significant changes were noted during the study. The available information in humans is inadequate for MRL derivation.

Studies in animals provide information on lethality and a wide range of end points. The lowest lowest-observed-adverse-effect level (LOAEL) in an acute-duration study was 15 mg 2,4-D/kg/day for behavioral alterations and decreased serum prolactin levels in rats (Stürtz et al. 2008). In that study, rats were administered 2,4-D mixed in the food on postpartum days 1–7. During this time, specific maternal behaviors were monitored and quantified. After the last observation period, the rats were killed and blood was collected for analysis of prolactin. The brain was removed and endogenous monoamines were determined in the arcuate nucleus. The study reported that exposure to ≥15 mg 2,4-D/kg/day (lowest dose tested) significantly increased latency of retrieval of pups, increased latency of crouching, decreased percent dams licking the pups, decreased percent dams licking the anogenital region of the pups, increased percent of dams leaving the nest, and increased time spent out of the nest. In addition exposure to 2,4-D significantly decreased serum prolactin levels compared to controls. Biochemical analyses of the arcuate nucleus showed decreased serotonin at ≥15 mg/kg/day and increased dopamine at ≥25 mg/kg/day. Information regarding the body weight of the pups was not provided.

Long-term oral studies suggest that the kidney is a target for 2,4-D toxicity; however, only one acute-duration study conducted microscopic examinations of the kidneys. Steiss et al. (1987) reported no significant histological alterations in the kidneys from dogs dosed once with 125 mg 2,4-D/kg in a capsule (highest dose tested). Two studies defined LOAELs of 50 mg/kg/day. In one of these studies, doses of 50 mg 2,4-D/kg/day (lowest dose tested) induced significant weight loss in pregnant Wistar rats when administered by gavage in water on gestation days (GDs) 6–15 (Fofana et al. 2000). It is not totally clear, however, whether the investigators meant that the final weight was lower than the starting weight or whether treated rats just gained less weight than control rats. In another developmental study,

administration of 50 mg 2,4-D/kg/day by gavage in corn oil to pregnant Sprague-Dawley rats also on GDs 6–15 did not affect maternal weight (terminal weight similar in treated and controls), but induced a statistically significant reduction in fetal weight (approximately 7%) measured on GD 20 and increased the incidence of some soft-tissue anomalies and skeletal malformations; the NOAEL was 25 mg 2,4-D/kg/day (Schwetz et al. 1971).

Data from Stürtz et al. (2008) could be considered for MRL derivation, specifically, the reduction in maternal serum prolactin levels or some of the altered maternal behaviors. For both end points, the lowest dose of 2,4-D tested, 15 mg/kg/day, was a LOAEL. However, in a subsequent study, the same group of investigators reported that exposure of adult rats to dietary doses of 2,4-D ranging from 2.5 to 70 mg/kg/day on postpartum days 1–16 resulted in significantly reduced pup body weight during the first 16 days of life (Stürtz et al. 2010). Statistically significant differences with controls were seen beginning on postnatal day (PND) 7 at maternal doses ≥5 mg/kg/day. From PND 10 on, even the lowest maternal dose of 2,4-D tested, 2.5 mg/kg/day, induced a significant reduction in pup weight relative to the control group, thus making this dose level a LOAEL for acute-exposure duration. Because the various data sets for body weight changes on PNDs 10–14 did not show clear dose-response relationships, attempts to derive an acute-duration oral MRL from any one of these data sets using benchmark dose (BMD) analysis were unsuccessful. Therefore, it is recommended that the intermediate-duration oral MRL of 0.009 mg 2,4-D/kg/day, which was derived by performing BMD analysis of the pup body weight data for PND 16 (see below), also be adopted as acute-duration oral MRL for 2,4-D.

- An MRL of 0.009 mg/kg/day has been derived for intermediate-duration oral exposure (15–364 days) to 2,4-D.

The MRL is based on a $BMDL_{RD05}$ (benchmark dose lower bound, 5% change from control) of 0.93 mg 2,4-D/kg/day for decreased body weight in rat's offspring on PND 16 (Stürtz et al. 2010). No human data were located. The database for animals is extensive and suggests that the kidney is a target organ for 2,4-D toxicity. Dogs appeared to be more sensitive than rodents, and as mentioned earlier, this seems due to dogs having a significantly lower capacity to eliminate 2,4-D via the kidneys than other species, including humans (Timchalk 2004). Therefore, dogs might not be a relevant species for evaluation of human health risk, and will not be considered for MRL derivation. The lowest LOAEL identified among intermediate-duration oral studies is 2.5 mg 2,4-D/kg/day for alterations in milk ejection in rat dams and reduced postnatal pup weight during maternal exposure to 2,4-D in the food on postpartum days 1–16 (Stürtz et al. 2010). Reduced offspring body weight was also reported in other studies in which rat dams were exposed to 2,4-D for longer periods that also included postpartum, although at higher estimated

maternal doses. In a 2-generation reproductive study, pup body weight was reduced significantly on PND 28 at estimated maternal doses ≥35 mg 2,4-D/kg/day during lactation, but not at 10 mg 2,4-D/kg/day (EPA 1986). Marty et al. (2013) reported significantly reduced pup weight (about 10%) on PND 22 at estimated maternal doses of approximately 9 mg 2,4-D/kg/day during lactation, but lower doses were not tested. In a 3-generation study, reduced pup weight was noted at maternal doses of approximately 111 mg 2,4-D/kg/day, but not 37 mg/kg/day (Hansen et al. 1971). The reasons for the apparent discrepancy regarding maternal dose levels at which offspring weight is significantly affected are not clear, but could be related to the different manners of estimating maternal intake of test material.

Several studies reported histological alterations in the kidneys from rats following exposure to 2,4-D; the results are summarized in Table 2-1. As the table shows, there is considerable dispersion of the data. The lowest dose at which alterations were reported (as described in the report reviewed) is 5 mg 2,4-D/kg/day (incidence significantly different from controls) for increased brown pigmentation in tubular cells in male and female F-344 rats (EPA 1985). Degenerative changes were reported at 45 and 60 mg 2,4-D/kg/day in male and female F-344 rats (EPA 1984; Gorzinski et al. 1987) and at 25 and 45 mg 2,4-D/kg/day in male Sprague-Dawley rats (Marty et al. 2013; Saghir et al. 2013). Simple hyperplasia was reported in male Sprague-Dawley rats dosed with approximately 7.1 mg 2,4-D/kg/day for 13 weeks (Ozaki et al. 2001). In another 13-week study, Charles et al. (1996a) stated that histological alterations were seen predominantly at 300 mg acid equivalents/kg/day and consisted of brush border loss in proximal tubular cells and vacuolization of kidney tubular cells in both male and female F-344 rats, suggesting that the NOAEL was 100 mg/kg/day. However, no kidney lesions were listed for 2,4-D acid in Table 1 of the study, suggesting that none occurred or the incidence in the treated groups was not significantly different than in the control group.

In B6C3F1 mice, exposure to ≥15 mg 2,4-D/kg/day for 13 weeks resulted in increased incidence of homogeneity and altered tinctorial properties of the cytoplasm of renal epithelial cells and decreased intracellular/intraluminal vacuolization in the kidney cortex of males (EPA 1984). It is unclear whether these changes were adverse or not. The same alterations were reported in male B6C3F1 mice exposed to ≥15 mg 2,4-D/kg/day for 52 weeks (EPA 1987a). In yet another 13-week study in B6C3F1 mice, exposure to approximately 430 mg 2,4-D/kg/day (highest dose tested) caused lesions in renal tubular epithelial cells characterized as simple hyperplasia; no changes were reported at approximately 180 mg 2,4-D/kg/day (Ozaki et al. 2001).

Table 2-1. Histological Alterations in Kidneys from Rats and Mice in Intermediate-Duration Studies

Study details	LOAEL (mg/kg/day)	NOAEL (mg/kg/day)	Results	Reference
F-344 rats, 13 weeks	300	100	Brush border loss in proximal tubular cells; vacuolization of kidney tubular cells (both sexes)	Charles et al. 1996a
F-344 rats, 13 weeks	45	15	Degenerative changes in kidneys (both sexes)	EPA 1984
F-344 rats, 52 weeks	5	1	Increased tubular cell (brown pigment) (both sexes)	EPA 1985
	15	5	Moderate fine vacuolization of cytoplasm of renal cortex (females)	
F-344 rats, 13 weeks	60	15	Slight multifocal degeneration of descending proximal tubules (both sexes)	Gorzinski et al. 1987
Sprague-Dawley rats, 2-generation	45	17	Slight degeneration of proximal convoluted tubules (F0 males)	Marty et al. 2013
Sprague-Dawley rats, 13 weeks	7.1	1.5	Simple hyperplasia (males)	Ozaki et al. 2001
F-344 rats, 2-generation	20	5	Increased focal nuclear density in medullary tubules (males)	EPA 1987b
Sprague-Dawley rats, 70 days	25	6	Slight degenerative multifocal lesions in proximal convoluted tubules (males)	Saghir et al. 2013
B6C3F1 mice, 13 weeks	15	5	Increased homogeneity and altered tinctorial properties of cytoplasm; decreased intracellular vacuolization in cortex (males)	EPA 1984
B6C3F1 mice, 52 weeks	15	1	Increased cytoplasmic homogeneity; decreased cytoplasmic vacuolization in tubular epithelium (males)	EPA 1987a
B6C3F1 mice, 13 weeks	430	179	Simple hyperplasia (males)	Ozaki et al. 2001

LOAEL = lowest-observed-adverse-effect level; NOAEL = no-observed-adverse-effect level

Because of the inconsistencies between studies and the uncertainty in determining whether or not certain histological alterations in the kidneys from rats and mice should be considered adverse, these data were not considered for MRL derivation. Instead, data for body weight changes in rat offspring exposed to 2,4-D through maternal milk reported in the Stürtz et al. (2010) study were selected for derivation of an intermediate-duration oral MRL for 2,4-D.

As previously mentioned, in the Stürtz et al. (2010) study, groups of female Wistar rats (6–8/group) were fed a diet that provided 0, 2.5, 5, 10, 15, 25, 50, or 70 mg/kg/day 2,4-D (98% pure) on postpartum days 1–16. Dams were checked daily for clinical signs and food consumption and body weight were monitored. Milk ejection was assessed by changes in body weight of the pups after allowing the pups to suckle during 15-minute periods on postpartum days 11–13. Blood was collected on postpartum day 12 for determination of growth hormone, prolactin, and oxytocin. Dams were sacrificed on postpartum day 16, and the arcuate nucleus and the anterior lobe of the pituitary were isolated for biochemical analyses of monoamines and metabolites in the 15, 25, and 50 mg/kg/day dose groups. Maternal exposure to 2,4-D did not affect maternal body weight, and no pups died during the test period. Maternal exposure to 2,4-D significantly reduced pup weight beginning on PND 7 in all exposed groups except the lowest dose group; this group showed a significant reduction in body weight beginning on PND 10. Milk ejection was significantly reduced in all treated groups on postpartum day 13 by >50%, reaching approximately 75% reduction in the highest dose group. An injection of oxytocin to the dams partially restored milk production, indicating that 2,4-D, at least in part, inhibited oxytocin release, but not the capacity of the mammary gland to produce or secrete milk. Serum prolactin appeared to be reduced in all treated groups, although Figure 3A in the study does not indicate statistically significant differences between the controls and exposed groups. Serum oxytocin was significantly reduced at ≥25 mg 2,4-D/kg/day. Serotonin was significantly reduced in the arcuate nucleus at ≥15 mg 2,4-D/kg/day and dopamine was significantly increased at ≥25 mg/kg/day. Dopamine was also increased in the anterior pituitary at ≥15 mg 2,4-D/kg/day.

The offspring body weight data on PND16 were fit to all available continuous models in EPA's Benchmark Dose Software (BMDS, version 2.4.0) using a benchmark response (BMR) of 5% change from control. Although there are no established guidelines as to what minimal change in a continuous end point such as body weight is biologically significant, a 10% change is generally used for adult body weight. However, because fetal or neonatal organisms may be more susceptible than adults, a 5% change was deemed appropriate.

Because no models fit the complete dataset, first the highest dose and subsequently the next highest dose were dropped. Only two BMD models (Exponential model 4 and Hill model) provided an adequate fit by the various statistical criteria. Because the $BMDL_{RD05}$ estimates were sufficiently close, the model with the lowest Akaike's Information Criterion (AIC) (Exponential model 4) was selected. The Exponential model calculated BMD_{RD05} and $BMDL_{RD05}$ values of 1.27 and 0.93 mg 2,4-D/kg/day, respectively, for decreased pup body weight on PND 16. Dividing the $BMDL_{RD05}$ of 0.93 mg/kg/day by an uncertainty factor of 100 (10 for animal to human extrapolation and 10 for human variability) yields an intermediate-duration oral MRL of 0.009 mg/kg/day for 2,4-D. Further details of the MRL derivation are presented in Appendix A.

A chronic-duration oral MRL for 2,4-D was not derived, as explained below. No adequate human data were located. A limited number of chronic-duration oral studies in rats, mice, and dogs were available for review. These studies suggest that the kidney is a target for 2,4-D toxicity in mice. As noted earlier, dogs might not be a relevant species for evaluation of human health risk due to their significantly lower capacity to eliminate 2,4-D via the kidneys; thus, dogs were not considered a suitable species for MRL derivation (see Section 3.5.1). A 2-year bioassay in F-344 rats defined an overall NOAEL of 5 mg 2,4-D/kg/day for organs and tissue histopathology and hematological and clinical chemistry parameters (Charles et al. 1996b). Exposure to 75 mg 2,4-D/kg/day decreased platelet and erythrocyte counts and hematocrit in females (results not shown), increased serum alanine aminotransferase (ALT) in males and decreased serum T4 in both sexes. Histological alterations were noted at 150 mg 2,4-D/kg/day and consisted of a nonsignificant increase in parafollicular cell nodular hyperplasia in the thyroid from females and minimal panlobular tinctorial properties in the liver from males and females. No clear treatment-related histological alterations were observed in the kidneys. An earlier study did not report treatment-related alterations in organs and tissues from Osborne-Mendel rats dosed with up to approximately 92 mg 2,4-D/kg/day in the diet for 2 years (Hansen et al. 1971).

In B6C3F1 mice, exposure to 15 mg 2,4-D/kg/day for 2 years significantly increased the incidence of cytoplasmic homogeneity in the renal tubular epithelium from male mice; this was attributed to a reduction of cytoplasmic vacuoles normally present in the cytoplasm of epithelial cells (EPA 1987a). No significant increase was seen at 1 mg 2,4-D/kg/day. The same alterations were observed in the kidneys from male B6C3F1 mice dosed with ≥62.5 mg 2,4-D/kg/day in another 2-year study (Charles et al. 1996b); no significant increase occurred at 5 mg 2,4-D/kg/day. A significant increase in minimal degeneration with regeneration of the descending portion of the proximal tubules in male mice occurred with an incidence of 0/50 (control), 0/50 (5 mg/kg/day), 25/50 (62.5 mg/kg/day), and 48/50

(125 mg/kg/day), defining a NOAEL of 5 mg 2,4-D/kg/day and a LOAEL of 62.5 mg 2,4-D/kg/day for renal effects in this study (Charles et al. 1996b). No other treatment-related histological alterations in organs or tissues or in hematology tests were reported in mice in these studies. Because of the unclear biological significance of the reduced vacuolization of the cytoplasm in tubular cells in male B6C3F1 mice, the degeneration/regeneration change in the proximal tubule of male mice reported by Charles et al. (1996b) seemed to be a more toxicologically relevant end point for MRL derivation.

The incidence data for degeneration with regeneration of the descending portion of the proximal tubules in male mice were analyzed using all available dichotomous models in the EPA BMDS (version 2.4.0) using the extra risk option. Adequate model fit was judged by three criteria: goodness-of-fit p-value ($p>0.1$), visual inspection of the dose-response curve, and scaled residual at the data point (except the control) closest to the predefined BMR. Among all of the models providing adequate fit to the data, the lowest BMDL (95% lower confidence limit on the BMD) was selected as the point of departure (POD) when the difference between the BMDLs estimated from these models was >3-fold; otherwise, the BMDL from the model with the lowest Akaike information criterion (AIC) was chosen. All models except the Multistage (1-degree) model provided an adequate fit to the dataset. The model selected based on the criteria mentioned above was the Multistage (2-degree) model, which defined a BMD_{10} of 23.59 mg 2,4-D/kg/day and a $BMDL_{10}$ of 16.66 mg 2,4-D/kg/day. Dividing the $BMDL_{10}$ of 16.66 mg/kg/day by an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability) would yield a chronic-duration oral MRL of 0.2 mg/kg/day for 2,4-D. However, this value is higher than the intermediate-duration oral MRL of 0.009 mg /kg/day for 2,4-D. Therefore, it is recommended that a chronic-duration oral MRL for 2,4-D not be derived at this time. The intermediate-duration oral MRL is protective for chronic-duration exposure.

3. HEALTH EFFECTS

3.1 INTRODUCTION

The primary purpose of this chapter is to provide public health officials, physicians, toxicologists, and other interested individuals and groups with an overall perspective on the toxicology of 2,4-D. It contains descriptions and evaluations of toxicological studies and epidemiological investigations and provides conclusions, where possible, on the relevance of toxicity and toxicokinetic data to public health.

Most of the information available regarding exposure to 2,4-D and health end points in humans comes from studies of individuals occupationally exposed either through farming activities or manufacture, formulation, or packaging of herbicide products containing 2,4-D. In these activities, exposure is likely to be predominantly by dermal contact with products containing 2,4-D, with inhalation exposure playing a lesser role. Therefore, studies of humans involved in these activities are summarized in Section 3.2.3, Dermal Exposure. However, the reader should keep in mind that the health outcomes described are the result of exposure through multiple routes, usually a combination of inhalation, oral, and dermal. It is important to keep in mind that although most human exposures are to chemical mixtures containing 2,4-D, exposure to 2,4-D is the common factor between the studies.

This profile discusses 2,4-D and simple salts (e.g., sodium, ammonium) as representatives of the various forms present in commercial formulations.

A glossary and list of acronyms, abbreviations, and symbols can be found at the end of this profile.

3.2 DISCUSSION OF HEALTH EFFECTS BY ROUTE OF EXPOSURE

To help public health professionals and others address the needs of persons living or working near hazardous waste sites, the information in this section is organized first by route of exposure (inhalation, oral, and dermal) and then by health effect (e.g., death, systemic, immunological, neurological, reproductive, developmental, and carcinogenic effects). These data are discussed in terms of three exposure periods: acute (14 days or less), intermediate (15–364 days), and chronic (365 days or more).

Levels of significant exposure for each route and duration are presented in tables and illustrated in figures. The points in the figures showing no-observed-adverse-effect levels (NOAELs) or lowest-observed-adverse-effect levels (LOAELs) reflect the actual doses (levels of exposure) used in the studies.

LOAELs have been classified into "less serious" or "serious" effects. "Serious" effects are those that evoke failure in a biological system and can lead to morbidity or mortality (e.g., acute respiratory distress or death). "Less serious" effects are those that are not expected to cause significant dysfunction or death, or those whose significance to the organism is not entirely clear. ATSDR acknowledges that a considerable amount of judgment may be required in establishing whether an end point should be classified as a NOAEL, "less serious" LOAEL, or "serious" LOAEL, and that in some cases, there will be insufficient data to decide whether the effect is indicative of significant dysfunction. However, the Agency has established guidelines and policies that are used to classify these end points. ATSDR believes that there is sufficient merit in this approach to warrant an attempt at distinguishing between "less serious" and "serious" effects. The distinction between "less serious" effects and "serious" effects is considered to be important because it helps the users of the profiles to identify levels of exposure at which major health effects start to appear. LOAELs or NOAELs should also help in determining whether or not the effects vary with dose and/or duration, and place into perspective the possible significance of these effects to human health.

The significance of the exposure levels shown in the Levels of Significant Exposure (LSE) tables and figures may differ depending on the user's perspective. Public health officials and others concerned with appropriate actions to take at hazardous waste sites may want information on levels of exposure associated with more subtle effects in humans or animals (LOAELs) or exposure levels below which no adverse effects (NOAELs) have been observed. Estimates of levels posing minimal risk to humans (Minimal Risk Levels or MRLs) may be of interest to health professionals and citizens alike.

A User's Guide has been provided at the end of this profile (see Appendix B). This guide should aid in the interpretation of the tables and figures for Levels of Significant Exposure and the MRLs.

3.2.1 Inhalation Exposure

Most of the information available regarding exposure to 2,4-D and health end points in humans comes from studies of individuals occupationally exposed either through farming activities or manufacture, formulation, or packaging of herbicide products containing 2,4-D. In these activities, exposure is likely to be predominantly by dermal contact with products containing 2,4-D, with inhalation exposure playing a lesser role. Therefore, studies of humans involved in these activities are summarized in Section 3.2.3, Dermal Exposure. However, the reader should keep in mind that the health outcomes described are the result of exposure through multiple routes, usually a combination of inhalation, oral, and dermal. It is

important to keep in mind that although most human exposures are to chemical mixtures containing 2,4-D, exposure to 2,4-D is the common factor between the studies.

Information available regarding health effects in animals following inhalation exposure was limited to a report of deaths in rats and a 28-day inhalation study in rats that examined a wide range of end points (EPA 2008). The study also included observations during a recovery period.

3.2.1.1 Death

An inhalation LC_{50} >1,790 mg/m^3 was reported for 2,4-D in rats (EPA 2005a); no further details were provided. No deaths were reported among Sprague-Dawley rats exposed nose-only to ≤1,000 mg/m^3 2,4-D dusts 6 hours/day, 5 days/week for 28 days (EPA 2008).

3.2.1.2 Systemic Effects

The highest NOAEL values and all LOAEL values from the EPA (2008) study for systemic effects are recorded in Table 3-1 and plotted in Figure 3-1.

Respiratory Effects. Labored breathing was reported in rats exposed intermittently nose-only to 1,000 mg/m^3 2,4-D dust in a 28-day inhalation study (EPA 2008). The effect was first seen on the 12th exposure; no such effect was seen in rats exposed to ≤300 mg/m^3 2,4-D. Microscopic examination of the respiratory tract of the rats at termination showed lesions restricted to the larynx in all exposed groups (50, 100, 300, and 1,000 mg/m^3 2,4-D). The lesions consisted of squamous/squamoid epithelial metaplasia with hyperkeratosis, hyperplasia of the arytenoid epithelium, and increased number of mixed inflammatory cells and showed dose-related severity. Examination of rats from the highest exposure group during a 4-week recovery period showed that the lesions persisted, but with reduced severity.

Cardiovascular Effects. No gross or microscopic lesions were reported in the heart or thoracic aorta from rats intermittently exposed nose-only to ≤1,000 mg/m^3 2,4-D dusts for 28 days (EPA 2008).

Gastrointestinal Effects. Intermittent nose-only exposure of rats to ≤1,000 mg/m^3 2,4-D dusts for 28 days did not induce gross or microscopic lesions in the gastrointestinal tract, including the pancreas (EPA 2008).

Table 3-1 Levels of Significant Exposure to 2,4-Dichlorophenoxyacetic Acid - Inhalation

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/m³)	LOAEL Less Serious (mg/m³)		LOAEL Serious (mg/m³)		Reference Chemical Form	Comments
INTERMEDIATE EXPOSURE										
Systemic										
1	Rat (Sprague-Dawley)	28 d 5 d/wk 6 hr/d	Resp		50	(squamous epithelial hyperplasia and metaplasia in larynx)	1000	(labored breathing)	EPA 2008 2,4-dichlorophenoxyacetic acid	NOAELs are for histopathology.of organs.
			Cardio	1000						
			Gastro	1000						
			Hemato	100	300	(20-26% decrease in reticulocytes)				
			Musc/skel	1000						
			Hepatic	100 F	300 F	(24% increased serum alkaline phosphatase)				
			Renal	1000						
			Endocr	1000						
			Dermal	1000						
			Ocular	1000						
			Bd Wt	300 F	1000 F	(11-13% reduced body weight during recovery)				
			Metab	1000						
Immuno/ Lymphoret										
2	Rat (Sprague-Dawley)	28 d 5 d/wk 6 hr/d		1000					EPA 2008 2,4-dichlorophenoxyacetic acid	NOAEL is for histopathology of lymphoreticular tissues.

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Table 3-1 Levels of Significant Exposure to 2,4-Dichlorophenoxyacetic Acid - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/m³)	LOAEL Less Serious (mg/m³)	LOAEL Serious (mg/m³)	Reference Chemical Form	Comments
Neurological								
3	Rat (Sprague-Dawley)	28 d 5 d/wk 6 hr/d		1000			EPA 2008 2,4-dichlorophenoxyacetic acid	NOAEL is for histopathology of brain, spinal cord, and peripheral nerves.
Reproductive								
4	Rat (Sprague-Dawley)	28 d 5 d/wk 6 hr/d		1000			EPA 2008 2,4-dichlorophenoxyacetic acid	NOAEL is for histopathology of reproductive organs.

a The number corresponds to entries in Figure 3-1.

Bd Wt = body weight; Cardio = cardiovascular; d = day(s); Endocr = endocrine; F = Female; Gastro = gastrointestinal; Hemato = hematological; hr = hour(s); Immuno/Lymphoret = immunological/lymphoreticular; LOAEL = lowest-observed-adverse-effect level; Metab = metabolism; Musc/skel = musculoskeletal; NOAEL = no-observed-adverse-effect level; Resp = respiratory; wk = week(s)

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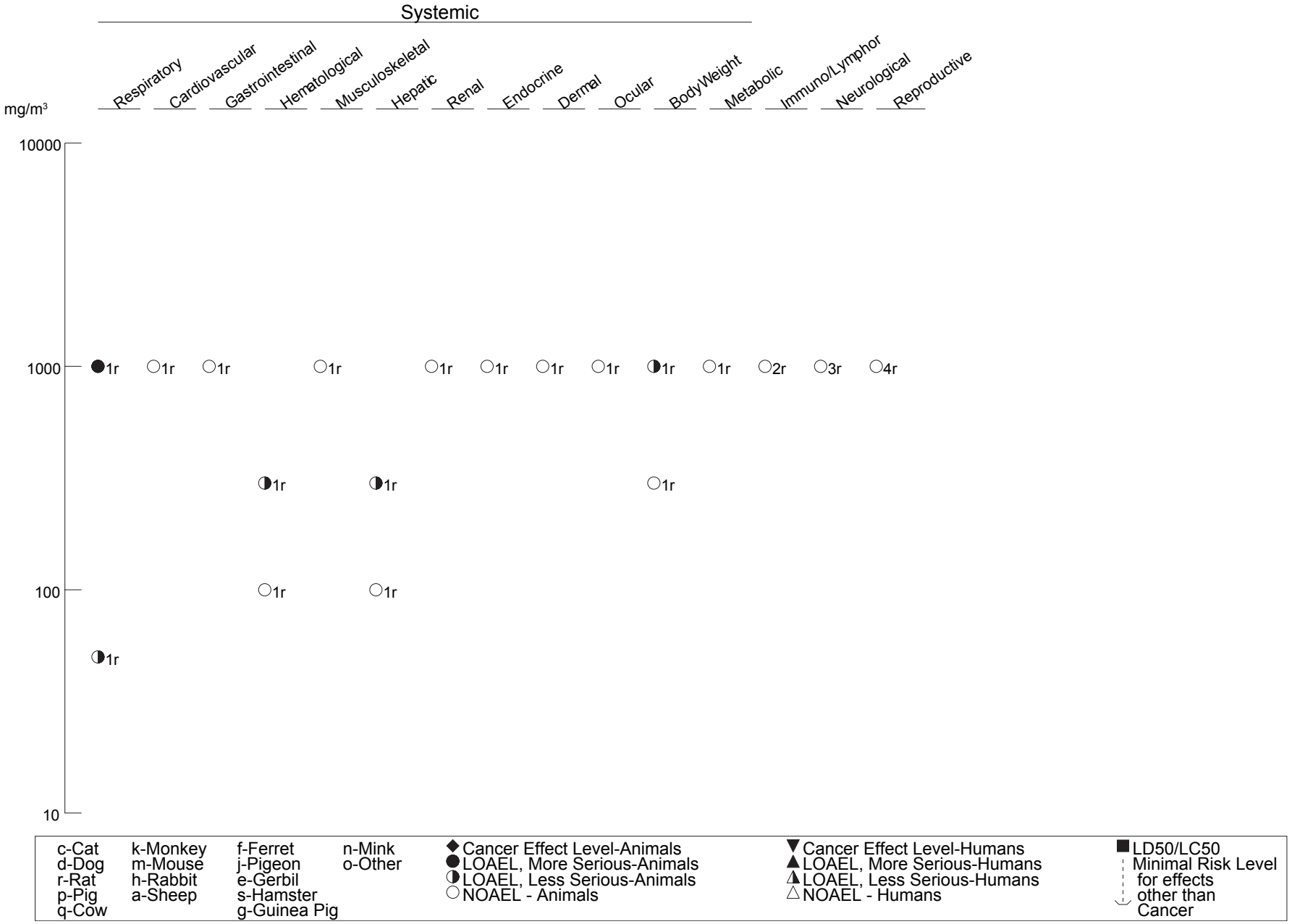
Figure 3-1 Levels of Significant Exposure to 2,4-Dichlorophenoxyacetic Acid - Inhalation

Intermediate (15-364 days)



Hematological Effects. Hematology tests conducted on male and female rats intermittently exposed nose-only to ≥300 mg/m^3 2,4-D dusts for 28 days showed a significant decrease (20–26%) in reticulocytes (EPA 2008). This effect persisted during a 4-week recovery period in females exposed to 1,000 mg/m^3 2,4-D dusts. The study also reported a reversible decrease in leukocyte counts (~31%) in female rats exposed to 1,000 mg/m^3 2,4-D dusts. However, because this did not occur in males, pre-exposure values were not established, and there was no correlating pathology, it was not considered toxicologically significant.

Musculoskeletal Effects. Intermittent nose-only exposure of rats to ≤1,000 mg/m^3 2,4-D dusts for 28 days did not induce gross or microscopic lesions in bone or skeletal muscle (EPA 2008).

Hepatic Effects. Female rats intermittently exposed nose-only to 1,000 mg/m^3 2,4-D dusts for 28 days had a significant increase in serum alkaline phosphatase activity (40%) and aspartate aminotransferase activity (35%) relative to controls at termination of exposure (EPA 2008). Females exposed to 300 mg/m^3 2,4-D dusts also showed a significant increase in alkaline phosphatase activity (24%). These values tended to return to control levels at the end of a 4-week recovery period; no significant effects were reported at 100 mg/m^3 2,4-D. Male rats exposed to 1,000 mg/m^3 2,4-D showed a significant increase in serum alanine aminotransferase activity at termination of exposure, which appeared to be due to an outlier value nearly 4 times greater than the other values. No other treatment-related alterations in clinical chemistry parameters used to assess liver function were reported. Gross and microscopic examination of the liver did not show treatment-related alterations.

Renal Effects. Intermittent nose-only exposure of rats to ≤1,000 mg/m^3 2,4-D dusts for 4 weeks did not induce gross or microscopic alterations in the kidneys (EPA 2008). Serum creatinine and blood urea nitrogen (BUN) values were also not significantly affected by exposure to 2,4-D. No urinalysis was performed in the study.

Endocrine Effects. Gross and microscopic examination of the pituitary, adrenal, thyroid, and parathyroid glands from rats exposed nose-only to ≤1,000 mg/m^3 2,4-D dusts intermittently for 28 days did not reveal treatment-related alterations (EPA 2008).

Dermal Effects. Examination of the skin of rats exposed intermittently nose-only to ≤1,000 mg/m^3 2,4-D dusts for 28 days did not show gross lesions (EPA 2008).

Ocular Effects. Ophthalmoscopic examination of the eyes from rats intermittently exposed nose-only to ≤1,000 mg/m^3 2,4-D dusts for 28 days did not show changes compared to pre-exposure test results (EPA 2008). Chromodacryorrhea (red lacrimation caused by excessive secretion of porphyrins with tears) occurred on day 12 and intermittently thereafter.

Body Weight Effects. Body weight of female rats intermittently exposed nose-only to 1,000 mg/m^3 2,4-D dusts for 28 days followed by a 4-week recovery period was significantly reduced (11–13%) from day 14 onward relative to controls (EPA 2008). Food consumption in this group was reduced approximately 10% during the study. No significant effects were reported in females exposed to ≤300 mg/m^3 2,4-D. In males, differences between exposed and control groups were either not statistically significant or were ≤10%.

Metabolic Effects. Intermittent nose-only exposure of rats to ≤1,000 mg/m^3 2,4-D dusts for 28 days did not significant alter serum electrolytes or glucose levels (EPA 2008).

3.2.1.3 Immunological and Lymphoreticular Effects

Significant increases in absolute and relative (to body weight and brain) spleen weight occurred in male rats intermittently exposed nose-only to ≤1,000 mg/m^3 2,4-D dusts for 28 days and allowed to recover for 4 additional weeks (EPA 2008). In females, absolute spleen weight was significantly decreased after recovery. Because gross and microscopic examination of the spleen, thymus, and lymph nodes from exposed rats did not show treatment-related alterations, the biological significance of the changes in spleen weight are unknown and are not listed in Table 3-1.

The exposure concentration of 1,000 mg/m^3 is listed as a NOAEL for lymphoreticular effects in rats in Table 3-1 and plotted in Figure 3-1.

3.2.1.4 Neurological Effects

No treatment-related gross or microscopic alterations were reported in the brain, spinal cord, or peripheral nerves from rats intermittently exposed nose-only to ≤1,000 mg/m^3 2,4-D dusts for 28 days (EPA 2008).

The NOAEL value for neurological effects in rats from EPA (2008) is recorded in Table 3-1 and plotted in Figure 3-1.

3.2.1.5 Reproductive Effects

Gross and microscopic examination of primary or secondary reproductive organs of male and female rats intermittently exposed nose-only to ≤1,000 mg/m^3 2,4-D dusts for 28 days did not show treatment-related alterations (EPA 2008).

The NOAEL value for reproductive effects in rats from EPA (2008) is recorded in Table 3-1 and plotted in Figure 3-1.

3.2.1.6 Developmental Effects

No studies were located regarding developmental effects in animals following inhalation exposure to 2,4-D.

3.2.1.7 Cancer

No studies were located regarding cancer in humans or animals following inhalation exposure to 2,4-D.

3.2.2 Oral Exposure

As previously mentioned, most of the information available regarding exposure to 2,4-D and health end points in humans comes from studies of individuals occupationally exposed either through farming activities or manufacture, formulation, or packaging of herbicide products containing 2,4-D. In these activities, exposure is likely to be predominantly by dermal contact with products containing 2,4-D, with inhalation exposure playing a lesser role. Therefore, studies of humans involved in these activities are summarized in Section 3.2.3, Dermal Exposure.

Information regarding oral exposure to 2,4-D in humans comes mainly from case reports of intentional or accidental ingestion of commercial herbicide formulations. Because most of these products also contained other ingredients that can be toxic (i.e., organic solvents, kerosene-like solvents or other herbicides), health outcomes observed following exposure cannot totally be attributed to 2,4-D. Additional information regarding clinical features of acute exposure to chlorophenoxy herbicides can be found in Bradberry et al. (2000).

3.2.2.1 Death

There have been deaths reported after intentional or accidental ingestion of products containing 2,4-D. Some examples are summarized below.

Nielsen et al. (1965) reported the case of a man who ingested an unknown amount of a commercial preparation containing the dimethyl amine salt of 2,4-D and died. An autopsy conducted on the same day of death showed acute congestion in all internal organs. Histological examination of the nervous system at various levels showed severe, degenerative changes of ganglion cells. Spots of acute emphysema were reported in the lungs, whereas the bronchioles contained presumed aspirated material. The total amount of 2,4-D measured in the various organs, blood, and urine was approximately 6 g (~80 mg/kg body weight). Dudley and Thapar (1972) reported the case of a man who died 6 days after ingestion of an unknown amount of 2,4-D. Signs observed prior to death included deep coma, altered respiration, hyperactive deep tendon reflexes, and moderate emphysema. Death was presumed to have been due to atrial fibrillation induced by muscle irritability associated with 2,4-D ingestion. Microscopic examination of tissues showed lesions in the brain, lungs, liver, and kidneys. Because the subject was 76 years old and autopsy was delayed for 36 hours, many of the histopathological alterations observed may not have been necessarily due to exposure to 2,4-D. Smith and Lewis (1987) reported a lethal case to have been due to ingestion of an unknown amount of an herbicide containing 2,4-D, based on the large amounts of 2,4-D found in the stomach and liver. No information was available regarding signs or symptoms preceding death. The only reported pathological findings were pulmonary edema and reddish watery fluid in the abdominal and thoracic cavities. An additional case of oral intoxication that ended up in death was reported by Keller et al. (1994). In this case, the subject had intentionally ingested an unknown amount of a commercial product that contained 500 g of 2,4-D/L. Based on levels of 2,4-D in blood, the investigators estimated that the amount of 2,4-D ingested was at least 25–35 g. Respiratory and kidney failure developed; death occurred after 48 hours of intensive care due to multiple organ failure.

Studies in rats have reported oral LD_{50} values between 600 and 800 mg/kg for 2,4-D (Elo et al. 1988; Gorzinski et al. 1987; Hill and Carlisle 1947). In one study, males appeared to be slightly more sensitive than females (Gorzinski et al. 1987). An early study that tested various species reported oral LD_{50} values for 2,4-D sodium salt of 1,000, 800, 666, and 375 mg/kg for guinea pigs, rabbits, rats, and mice, respectively (Hill and Carslisle 1947); it was also reported that the sodium and ammonium salts had about the same toxicity as the acid. In a developmental study, repeated doses of 115 mg/kg 2,4-D decreased survival of pregnant rats (Chernoff et al. 1990). An oral LD_{50} of 100 mg/kg was reported for 2,4-D in

mongrel dogs (Drill and Hiratzka 1953), although results from other acute studies in dogs do not support such a relatively low LD_{50} value (Dickow et al. 2000; Steiss et al. 1987). Common signs reported by Drill and Hiratzka (1953) included stiffness of the extremities with some muscular incoordination, lethargy, paralysis of the hindquarters, stupor, coma, and death. Hill and Carslisle (1947) noted that some combination of some of these signs resembled myotonia congenita.

In a repeated dose 13-week study, three out of four dogs administered capsules of 20 mg/kg/day 5 days/week died on days 18, 25, and 49 (Drill and Hiratzka 1953). Higher-than-normal muscle tonus in the hind limbs, particularly on passive extension, was described in these dogs; slight ataxia was also present. The days preceding death, the dogs showed difficulty in chewing or swallowing and there was also some oozing of blood from the gums and buccal mucosa.

LD_{50} values and lethal doses are presented in Table 3-2 and plotted in Figure 3-2.

3.2.2.2 Systemic Effects

The highest NOAEL values and all LOAEL values from each reliable study for systemic effects in each species and duration category are recorded in Table 3-2 and plotted in Figure 3-2.

Respiratory Effects. Tachypnea was reported in a person who drank 100–200 mL of a 40% solution of 2,4-D (40–80 g) (Durakovic et al. 1992). Emphysema in the lungs was reported in two lethal cases reported by Nielsen et al. (1965) and Dudley and Thapar (1972). A subject who ingested approximately 110 mg 2,4-D/kg from a commercial herbicide product complained of breathing difficulties 24 hours after admission to the hospital (Berwick 1970). Pulmonary edema was noted in a lethal case reported by Smith and Lewis (1987) and respiratory failure was noted in the case reported by Keller et al. (1994).

With one exception, studies in animals that conducted gross and microscopic examination of the respiratory tract did not report alterations attributed to exposure to 2,4-D. No significant effects were reported in an acute-duration study in dogs exposed once to ≤125 mg 2,4-D/kg (Steiss et al. 1987) and in intermediate-duration studies in rats exposed to ≤300 mg 2,4-D/kg/day (Charles et al. 1996a; EPA 1984, 1985; Gorzinski et al. 1987), mice exposed to ≤90 mg 2,4-D/kg/day (EPA 1984, 1987a), and dogs exposed to 7.5 mg 2,4-D/kg/day (Charles et al. 1996c).

Table 3-2 Levels of Significant Exposure to 2,4-Dichlorophenoxyacetic Acid - Oral

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
ACUTE EXPOSURE								
Death								
1	Rat (Sprague-Dawley)	10 d Gd 6-15 1 x/d (GO)				115 F (decreased survival)	Chernoff et al. 1990 2,4-dichlorophenoxyacetic acid	
2	Rat (Sprague-Dawley)	once (G)				600 M (LD50)	Elo et al. 1988 2,4-dichlorophenoxyacetic acid	
3	Rat (Fischer- 344)	once (GO)				639[b] M (LD50) 764 F (LD50)	Gorzinski et al. 1987 2,4-dichlorophenoxyacetic acid	
4	Rat White	once (GW)				666 (LD50)	Hill and Carlisle 1947 Sodium (2,4-dichlorophenoxy) acetate	
5	Rat (Fischer- 344)	once (GO)				500 (lethal dose)	Mattsson et al. 1997 2,4-dichlorophenoxyacetic acid	
6	Mouse White	once (GW)				375 (LD50)	Hill and Carlisle 1947 Sodium (2,4-dichlorophenoxy) acetate	
7	Gn Pig (NS)	once (GW)				1000 (LD50)	Hill and Carlisle 1947 Sodium (2,4-dichlorophenoxy) acetate	
8	Dog (Mongrel)	once (C)				100 (LD50)	Drill and Hiratzka 1953 2,4-dichlorophenoxyacetic acid	

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Table 3-2 Levels of Significant Exposure to 2,4-Dichlorophenoxyacetic Acid - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
9	Rabbit (NS)	once (GW)				800 (LD50)	Hill and Carlisle 1947 Sodium (2,4-dichlorophenoxy) acetate	
Systemic								
10	Rat (Fischer- 344)	10 d Gd 6-15 1 x/d (GW)	Bd Wt	75 F			Charles et al. 2001 2,4-dichlorophenoxyacetic acid	
11	Rat (Sprague-Dawley)	10 d Gd 6-15 1 x/d (GO)	Bd Wt		115 M (decreased weight gain during treatment)		Chernoff et al. 1990 2,4-dichlorophenoxyacetic acid	
12	Rat (Wistar)	9 d Gd 6-15 1 x/d (GW)	Bd Wt		50 F (weight loss during pregnancy)		Fofana et al. 2000 2,4-dichlorophenoxyacetic acid	
13	Rat (Fischer- 344)	once (GO)	Musc/skel	250			Mattsson et al. 1997 2,4-dichlorophenoxyacetic acid	NOAEL is for histopathology of the pituitary, retina, and skeletal muscle tissue.
			Endocr	250				
			Ocular	250				
			Bd Wt	250				
14	Rat (Sprague-Dawley)	Gd 6-15 10 d 1 x/d (GO)	Bd Wt	87.5 F			Schwetz et al. 1971 2,4-dichlorophenoxyacetic acid	

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Table 3-2 Levels of Significant Exposure to 2,4-Dichlorophenoxyacetic Acid - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
15	Rat (Wistar)	6 d (F)	Endocr		15 F (significant reduction in serum prolactin)		Sturtz et al. 2008 2,4-dichlorophenoxyacetic acid	
16	Mouse (ICR)	10 d Gd 0-9 (W)	Bd Wt	100 F			Dinamarca et al. 2007 2,4-dichlorophenoxyacetic acid	
17	Dog (Beagle)	once (C)	Gastro		200 F (vomiting and diarrhea)		Dickow et al. 2000 2,4-dichlorophenoxyacetic acid	
			Musc/skel		200 F (insertional myotonia)			
			Hepatic	200 F				
			Renal	200 F				
			Metab		200 F (reduced serum calcium and potassium)			
18	Dog (Mongrel)	once (C)	Resp	125 F			Steiss et al. 1987 2,4-dichlorophenoxyacetic acid	NOAELs are for organ histopathology.
			Cardio	125 F				
			Gastro	125 F				
			Musc/skel	125 F				
			Hepatic	125 F				
			Renal	125 F				

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Table 3-2 Levels of Significant Exposure to 2,4-Dichlorophenoxyacetic Acid - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
19	Rabbit (New Zealand)	13 d Gd 6-18 1 x/d (GW)	Bd Wt	90 F			Charles et al. 2001 2,4-dichlorophenoxyacetic acid	
Immuno/ Lymphoret								
20	Dog (Mongrel)	once (C)		125 F			Steiss et al. 1987 2,4-dichlorophenoxyacetic acid	NOAELs are for histopathology of lymph nodes and spleen.
Neurological								
21	Rat (Sprague-Dawley)	once (G)		150 M		300 M (vascular damage in the CNS)	Elo et al. 1988 2,4-dichlorophenoxyacetic acid	
22	Rat (Fischer- 344)	once (GO)		75	250 (altered gait and increased motor activity 1 day post-dosing)		Mattsson et al. 1997 2,4-dichlorophenoxyacetic acid	
23	Rat (Wistar)	6 d (F)			15 F (altered maternal behavior)		Sturtz et al. 2008 2,4-dichlorophenoxyacetic acid	
24	Dog (Mongrel)	once (C)		125 F			Steiss et al. 1987 2,4-dichlorophenoxyacetic acid	NOAEL is for no changes in nerve conduction velocity and histopathology of brain and spinal cord.

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Table 3-2 Levels of Significant Exposure to 2,4-Dichlorophenoxyacetic Acid - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
Reproductive								
25	Mouse (ICR)	10 d Gd 0-9 (W)		100 F			Dinamarca et al. 2007 2,4-dichlorophenoxyacetic acid	NOAEL is for no changes in corpora lutea, implantations and resorption sites.
Developmental								
26	Rat (Fischer- 344)	10 d Gd 6-15 1 x/d (GW)		75			Charles et al. 2001 2,4-dichlorophenoxyacetic acid	NOAEL is for no changes in litter data, fetal body weight and teratogenicity.
27	Rat (Sprague-Dawley)	10 d Gd 6-15 1 x/d (GO)			115 F (increased incidence of supernumerary ribs)		Chernoff et al. 1990 2,4-dichlorophenoxyacetic acid	
28	Rat (Wistar)	9 d Gd 6-15 1 x/d (GW)		50		70 (increased resorptions; renal malformations)	Fofana et al. 2000 2,4-dichlorophenoxyacetic acid	
29	Rat (Wistar)	10 d Gd 6-15 1 x/d (GW)				70 (lethality within first 2 weeks of life)	Fofana et al. 2002 2,4-dichlorophenoxyacetic acid	
30	Rat (Sprague-Dawley)	Gd 6-15 10 d 1 x/d (GO)		25 F	50 F (reduced fetal weight; increased incidence of soft-tissue and skeletal anomalies)		Schwetz et al. 1971 2,4-dichlorophenoxyacetic acid	

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Table 3-2 Levels of Significant Exposure to 2,4-Dichlorophenoxyacetic Acid - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
31	Mouse (CD-1)	5 d Gd 8-12 1 x/d (GO)			87.5 (reduced neonatal weight on postnatal day 1)		Kavlock et al. 1987 2,4-dichlorophenoxyacetic acid	
32	Hamster (Golden Syrian)	Gd 6-10 5 d 1x/d (GO)		100 F			Collins and Williams 1971 2,4-dichlorophenoxyacetic acid	NOAEL is for teratogenicity.
33	Rabbit (New Zealand)	13 d Gd 6-18 1 x/d (GW)		90			Charles et al. 2001 2,4-dichlorophenoxyacetic acid	
INTERMEDIATE EXPOSURE								
Death								
34	Dog (Mongrel)	13 wk 5 d/wk (C)				20 (3 out 4 dogs died on days 18, 25, and 49)	Drill and Hiratzka 1953 2,4-dichlorophenoxyacetic acid	
Systemic								
35	Rat (Wistar)	28 d Gd 16-21 Pnd 1-23 (F)	Bd Wt		70 M (11% reduced body weight on Pnd 90)		Bortolozzi et al. 1999 2,4-dichlorophenoxyacetic acid	Offspring were dosed directly until Pnd 90.

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Table 3-2 Levels of Significant Exposure to 2,4-Dichlorophenoxyacetic Acid - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
36	Rat (Fischer- 344)	13 wk ad lib (F)	Resp	300			Charles et al. 1996a 2,4-dichlorophenoxyacetic acid	
			Cardio	300				
			Gastro	300				
			Hemato	15	100 (decreased platelets)			
			Musc/skel	300				
			Hepatic	100	300 (hepatocellular hypertrophy)			
			Renal	15	100 (increased relative kidney weight)			
			Endocr	15 F	100 F (decreased serum T3 and T4; adrenal cortex hypertrophy)			
			Ocular	100 F		300 F (cataracts)		
			Bd Wt	100		300 (38-57% reduced weight gain)		
			Metab	300				

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Table 3-2 Levels of Significant Exposure to 2,4-Dichlorophenoxyacetic Acid - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
37	Rat (Fischer- 344)	13 wk ad lib (F)	Resp	45			EPA 1984 2,4-dichlorophenoxyacetic acid	
			Cardio	45				
			Gastro	45				
			Hemato	45				
			Musc/skel	45				
			Hepatic	45				
			Renal	15	45 (degenerative changes in renal cortex)			
			Endocr	45				
			Ocular	45				
			Bd Wt	45				
			Metab	45				

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Table 3-2 Levels of Significant Exposure to 2,4-Dichlorophenoxyacetic Acid - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
38	Rat (Fischer- 344)	52 wk ad lib (F)	Resp	45			EPA 1985 2,4-dichlorophenoxyacetic acid	
			Cardio	45				
			Gastro	45				
			Hemato	45				
			Musc/skel	45				
			Hepatic	45				
			Renal	1	5 (increased tubular cell brown pigment)			
			Endocr	45 M 5 F	15 F[b] (increased serum T4 on week 27)			
			Dermal	45				
			Ocular	45				
			Bd Wt	45				
			Metab	45				
39	Rat (Fischer- 344)	40 wk ad lib (F)	Hepatic	80			EPA 1986 2,4-dichlorophenoxyacetic acid	Hepatic NOAEL is for liver histopathology
			Bd Wt	80				

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Table 3-2 Levels of Significant Exposure to 2,4-Dichlorophenoxyacetic Acid - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
40	Rat (Fischer- 344)	40 wk ad lib (F)	Renal	5 M	20 M (histological alterations in renal tubules)		EPA 1987b 2,4-dichlorophenoxyacetic acid	
41	Rat (Fischer- 344)	13 wk ad lib (F)	Resp	150			Gorzinski et al. 1987 2,4-dichlorophenoxyacetic acid	
			Cardio	150				
			Gastro	150				
			Hemato	150				
			Musc/skel	150				
			Hepatic	100	150 (slight swelling and increased cytoplasmic homogeneity of hepatocytes)			
			Renal	15	60 (slight multifocal degeneration of descending proximal tubules)			
			Endocr	60 F	100 F (decreased serum T4)			
			Ocular	150				
			Bd Wt	100 F	150 F (21% decreased weight gain)			

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Table 3-2 Levels of Significant Exposure to 2,4-Dichlorophenoxyacetic Acid - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
42	Rat (CD)	M: 11 wk F: 10 wk ad lib (F)	Hemato	50 F			Marty et al. 2013 2,4-dichlorophenoxyacetic acid	
			Renal	16.6 M	45.3 M (slight degeneration of proximal convoluted tubules)			
			Endocr	25.1 F	50 F (decreased serum T3 and T4 and increased TSH on Gd 17)			
43	Rat (Fischer- 344)	52 wk ad lib (F)	Resp	75 F	150 F (pale foci in the lungs)		Mattsson et al. 1997 2,4-dichlorophenoxyacetic acid	NOAELs are for tissue histopathology.
			Musc/skel	150				
			Endocr	150				
			Ocular	75 F		150 F (retinal degeneration)		
			Bd Wt	75	150 (10% reduced terminal body weight)			
44	Rat (albino)	20 d GD 1-19 1 x/d (GO)	Bd Wt			100 F (40-54% reduced maternal weight gain during pregnancy)	Mazhar et al. 2014 2,4-dichlorophenoxyacetic acid	

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Table 3-2 Levels of Significant Exposure to 2,4-Dichlorophenoxyacetic Acid - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
45	Rat (Sprague-Dawley)	3 mo ad lib (F)	Hepatic	215 M			Ozaki et al. 2001 2,4-dichlorophenoxyacetic acid	
			Renal	1.5 M	7.1 M (lesions in renal tubule epithelium)			
			Bd Wt	215 M				
46	Rat (Sprague-Dawley)	71-96 d ad lib (F)	Renal	6 M	25 M (very slight degenerative lesions in kidneys)		Saghir et al. 2013 2,4-dichlorophenoxyacetic acid	
			Bd Wt	100 M				
47	Rat (Fischer- 344)	5 wk 2 d/wk (GO)	Bd Wt	80 M			Squibb et al. 1983 2,4-dichlorophenoxyacetic acid	
48	Rat (Wistar)	Ppd 1-16 (F)	Endocr		2.5 F (reduced serum prolactin)		Sturtz et al. 2010 2,4-dichlorophenoxyacetic acid	Milk ejection was reduced in all treated groups.
49	Rat (Wistar)	Gd 14-21 Pnd 0-14 ad lib (W)	Hepatic		126 F (increased liver weight and serum transaminases; liver histopathology)		Troudi et al. 2012a 2,4-dichlorophenoxyacetic acid	
			Bd Wt	126 F				

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Table 3-2 Levels of Significant Exposure to 2,4-Dichlorophenoxyacetic Acid - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
50	Mouse (B6C3F1)	13 wk ad lib (F)	Resp	90			EPA 1984 2,4-dichlorophenoxyacetic acid	
			Cardio	90				
			Gastro	90				
			Hemato	90				
			Musc/skel	90				
			Hepatic	90				
			Renal	5 M	15 M (histological alterations in renal cortex)			
			Endocr	90				
			Ocular	90				
			Bd Wt	90				

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Table 3-2 Levels of Significant Exposure to 2,4-Dichlorophenoxyacetic Acid - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
51	Mouse (B6C3F1)	52 wk ad lib (F)	Resp	45			EPA 1987a 2,4-dichlorophenoxyacetic acid	
			Cardio	45				
			Gastro	45				
			Hemato	45				
			Musc/skel	45				
			Hepatic	45				
			Renal	1 M 45 F	15 M[b] (reduced cytoplasmic vacuoles)			
			Endocr	45 F	1 M (decreased absolute and relative adrenals weight)			
			Dermal	45				
			Ocular	45				
			Bd Wt	45				
52	Mouse (B6C3F1)	3 mo ad lib (F)	Hepatic	429.4 M			Ozaki et al. 2001 2,4-dichlorophenoxyacetic acid	
			Renal	178.9 M	429.4 M (lesions in renal tubule epithelial cells)			
			Bd Wt	178.9 M	429.4 M (18% reduction in terminal body weight)			

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Table 3-2 Levels of Significant Exposure to 2,4-Dichlorophenoxyacetic Acid - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
53	Hamster (Golden Syrian)	3 mo ad lib (F)	Hepatic	474 M			Ozaki et al. 2001 2,4-dichlorophenoxyacetic acid	
			Renal	474 M				
			Bd Wt	474 M				
54	Dog (Beagle)	13 weeks ad lib (F)	Resp	7.5			Charles et al. 1996c 2,4-dichlorophenoxyacetic acid	
			Cardio	7.5				
			Gastro	7.5				
			Hemato	7.5				
			Musc/skel	7.5				
			Hepatic	3.75	7.5 (perivascular active inflammation in the liver)			
			Renal	1	3.75 (increased BUN and serum creatinine)			
			Endocr	7.5				
			Ocular	7.5				
			Bd Wt	7.5				
			Metab	7.5				

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Table 3-2 Levels of Significant Exposure to 2,4-Dichlorophenoxyacetic Acid - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
55	Dog (Beagle)	1 yr ad lib (F)	Resp	7.5			Charles et al. 1996c 2,4-dichlorophenoxyacetic acid	NOAELs are for tissue histopathology.
			Cardio	7.5				
			Gastro	7.5				
			Hemato	7.5				
			Musc/skel	7.5				
			Hepatic	1	5 (increased serum cholesterol; perivascular inflammation of liver)			
			Renal	1	5 (increased BUN and creatinine; tubular epithelium pigmentation)			
			Endocr	7.5				
			Ocular	7.5				
			Bd Wt	5 F		7.5 F (64% reducton in weight gain)		
			Metab	1	5 (decreased serum glucose)			
Immuno/ Lymphoret								
56	Rat (Fischer- 344)	13 wk ad lib (F)		300			Charles et al. 1996a 2,4-dichlorophenoxyacetic acid	NOAEL is for histopathology of lymphoreticular organs.

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Table 3-2 Levels of Significant Exposure to 2,4-Dichlorophenoxyacetic Acid - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
57	Rat (Fischer- 344)	13 wk ad lib (F)		45			EPA 1984 2,4-dichlorophenoxyacetic acid	NOAEL is for histopathology of spleen and thymus.
58	Rat (Fischer- 344)	52 wk ad lib (F)		45			EPA 1985 2,4-dichlorophenoxyacetic acid	NOAEL is for histopathology of lymphoreticular tissues.
59	Rat (Fischer- 344)	13 wk ad lib (F)		150			Gorzinski et al. 1987 2,4-dichlorophenoxyacetic acid	NOAEL is for histopathology of lymphoreticular tissues.
60	Rat (CD)	30 d (F)		75.3 M			Marty et al. 2013 2,4-dichlorophenoxyacetic acid	NOAEL is for no changes in humoral immune response and NK cell activity in F1 adult rats.
61	Rat (CD)	M: 11 wk F: 10 wk ad lib (F)		50 F			Marty et al. 2013 2,4-dichlorophenoxyacetic acid	NOAEL is for no changes in weight and histology of lymphoreticular organs.
62	Mouse (B6C3F1)	13 wk ad lib (F)		90			EPA 1984 2,4-dichlorophenoxyacetic acid	NOAEL is for histopathology of lymphoreticular tissues.

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Table 3-2 Levels of Significant Exposure to 2,4-Dichlorophenoxyacetic Acid - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
63	Mouse (B6C3F1)	52 wk ad lib (F)		45			EPA 1987a 2,4-dichlorophenoxyacetic acid	NOAEL is for histopathology of spleen, thymus and lymph nodes.
64	Dog (Beagle)	13 weeks ad lib (F)		7.5			Charles et al. 1996c 2,4-dichlorophenoxyacetic acid	NOAEL is for histopathology of lymphoreticular tissues.
65	Dog (Beagle)	1 yr ad lib (F)		7.5			Charles et al. 1996c 2,4-dichlorophenoxyacetic acid	NOAEL is for no changes in histopathology of lymphoreticular tissues.
Neurological								
66	Rat (Fischer- 344)	13 wk ad lib (F)		300			Charles et al. 1996a 2,4-dichlorophenoxyacetic acid	NOAEL is for histopathology of brain, spinal cord or sciatic nerve.
67	Rat (Fischer- 344)	13 wk ad lib (F)		45			EPA 1984 2,4-dichlorophenoxyacetic acid	NOAEL is for histopathology of the brain.
68	Rat (Fischer- 344)	52 wk ad lib (F)		45			EPA 1985 2,4-dichlorophenoxyacetic acid	NOAEL is for histopathology of central and peripheral nerve tissues.

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Table 3-2 Levels of Significant Exposure to 2,4-Dichlorophenoxyacetic Acid - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
69	Rat (Fischer- 344)	13 wk ad lib (F)		150			Gorzinski et al. 1987 2,4-dichlorophenoxyacetic acid	NOAEL is for histopathology of central and peripheral neural tissues.
70	Rat (CD)	20 d (F)		81.7 M			Marty et al. 2013 2,4-dichlorophenoxyacetic acid	NOAEL is for no neurobehavioral and neuropathological changes in adult F1 generation.
71	Rat (CD)	M: 11 wk F: 10 wk ad lib (F)		50 F			Marty et al. 2013 2,4-dichlorophenoxyacetic acid	NOAEL is for weight and histopathology of the brain.
72	Rat (Fischer- 344)	52 wk ad lib (F)		75	150 (increased forelimb grip strength)		Mattsson et al. 1997 2,4-dichlorophenoxyacetic acid	
73	Rat (Fischer- 344)	5 wk 2 d/wk (GO)			20 M (increased forelimb grip strength)		Squibb et al. 1983 2,4-dichlorophenoxyacetic acid	
74	Rat (Fischer- 344)	4 wk 7 d/wk (GO)		20 M	40 M (increased hindlimb grip strength)		Squibb et al. 1983 2,4-dichlorophenoxyacetic acid	

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Table 3-2 Levels of Significant Exposure to 2,4-Dichlorophenoxyacetic Acid - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
75	Mouse (B6C3F1)	13 wk ad lib (F)		90			EPA 1984 2,4-dichlorophenoxyacetic acid	NOAEL is for histopathology of brain, spinal cord, and sciatic nerve.
76	Mouse (B6C3F1)	52 wk ad lib (F)		45			EPA 1987a 2,4-dichlorophenoxyacetic acid	NOAEL is for histopathology of brain, spinal cord, and sciatic nerve.
77	Dog (Beagle)	13 weeks ad lib (F)		7.5			Charles et al. 1996c 2,4-dichlorophenoxyacetic acid	NOAEL is for histopathology of central and peripheral nervous system.
78	Dog (Beagle)	1 yr ad lib (F)		7.5			Charles et al. 1996c 2,4-dichlorophenoxyacetic acid	NOAEL is for histopathology of central and peripheral nervous system.
Reproductive								
79	Rat (Fischer- 344)	13 wk ad lib (F)		300			Charles et al. 1996a 2,4-dichlorophenoxyacetic acid	NOAEL is for histopathology of reproductive organs.
80	Rat (Fischer- 344)	13 wk ad lib (F)		45			EPA 1984 2,4-dichlorophenoxyacetic acid	NOAEL is for histopathology of reproductive organs.

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Table 3-2 Levels of Significant Exposure to 2,4-Dichlorophenoxyacetic Acid - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
81	Rat (Fischer- 344)	52 wk ad lib (F)		45			EPA 1985 2,4-dichlorophenoxyacetic acid	NOAEL is for histopathology of reproductive organs.
82	Rat (Fischer- 344)	40 wk ad lib (F)		80			EPA 1986 2,4-dichlorophenoxyacetic acid	NOAEL is for no changes in fertility and histopathology of ovaries and testes.
83	Rat (Fischer- 344)	13 wk ad lib (F)		150			Gorzinski et al. 1987 2,4-dichlorophenoxyacetic acid	NOAEL is for histopathology of reproductive organs of males and females.
84	Rat (Osborne-Mendel)	3-gen ad lib (F)		111			Hansen et al. 1971 2,4-dichlorophenoxyacetic acid	NOAEL is for no alterations in fertility.
85	Rat (albino)	30 d 1 x/d (GO)			50 M (decreased sperm count and motility; testes histopathology)		Joshi et al. 2012 2,4-dichlorophenoxyacetic acid	
86	Rat (CD)	M: 11 wk F: 10 wk ad lib (F)		45.3 M 50 F			Marty et al. 2013 2,4-dichlorophenoxyacetic acid	NOAEL is for no alterations in reproductive indices.

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Table 3-2 Levels of Significant Exposure to 2,4-Dichlorophenoxyacetic Acid - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
87	Rat (Sprague-Dawley)	71-96 d ad lib (F)		100			Saghir et al. 2013 2,4-dichlorophenoxyacetic acid	NOAEL is for no alterations in reproductive indices.
88	Mouse (B6C3F1)	13 wk ad lib (F)		90			EPA 1984 2,4-dichlorophenoxyacetic acid	NOAEL is for histopathology of reproductive organs.
89	Mouse (B6C3F1)	52 wk ad lib (F)		45			EPA 1987a 2,4-dichlorophenoxyacetic acid	NOAEL is for histopathology of reproductive organs.
90	Dog (Beagle)	13 weeks ad lib (F)		7.5			Charles et al. 1996c 2,4-dichlorophenoxyacetic acid	NOAEL is for histopathology of reproductive organs of males and females.
91	Dog (Beagle)	1 yr ad lib (F)		7.5			Charles et al. 1996c 2,4-dichlorophenoxyacetic acid	NOAEL is histopathology of reproductive organs.
Developmental								
92	Rat (Wistar)	28 d Gd 16-21 Pnd 1-23 (F)			70 (reduced preweaning pup's weight; neurobehavioral alterations in pups)		Bortolozzi et al. 1999 2,4-dichlorophenoxyacetic acid	

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Table 3-2 Levels of Significant Exposure to 2,4-Dichlorophenoxyacetic Acid - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
93	Rat (Fischer- 344)	40 wk ad lib (F)		10	35 (14-16% reduced pup body weight on Pnd 28)		EPA 1986 2,4-dichlorophenoxyacetic acid	
94	Rat (Osborne-Mendel)	3-gen ad lib (F)		37		111 (reduced pup's body weight and viability)	Hansen et al. 1971 2,4-dichlorophenoxyacetic acid	
95	Rat (CD)	M: 11 wk F: 10 wk ad lib (F)			9 F (reduced weight of pups on Pnd 22)		Marty et al. 2013 2,4-dichlorophenoxyacetic acid	
96	Rat (albino)	20 d GD 1-19 1 x/d (GO)				100 (31% reduced fetal weight; morphological and skeletal defects)	Mazhar et al. 2014 2,4-dichlorophenoxyacetic acid	
97	Rat (Sprague-Dawley)	71-96 d ad lib (F)		25	50 (decreased pup's weight on Pnd 14)		Saghir et al. 2013 2,4-dichlorophenoxyacetic acid	
98	Rat (Wistar)	Ppd 1-16 (F)			2.5[c] (significant reduction in postnatal pup's weight)		Sturtz et al. 2010 2,4-dichlorophenoxyacetic acid	

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Table 3-2 Levels of Significant Exposure to 2,4-Dichlorophenoxyacetic Acid - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
99	Rat (Wistar)	Gd 14-21 Pnd 0-14 ad lib (W)			126 (17% reduction in pup's weight; liver histopathology)		Troudi et al. 2012a 2,4-dichlorophenoxyacetic acid	Effects are on pups on Pnd 14.
100	Rat (Wistar)	Gd 14-21 Pnd 1-14 ad lib (W)			126 (17% reduced pup's weight on Pnd 14; bone histopathology)		Troudi et al. 2012b 2,4-dichlorophenoxyacetic acid	

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Table 3-2 Levels of Significant Exposure to 2,4-Dichlorophenoxyacetic Acid - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
CHRONIC EXPOSURE								
Systemic								
101	Rat (Fischer- 344)	2 yr ad lib (F)	Resp	150			Charles et al. 1996b 2,4-dichlorophenoxyacetic acid	NOAELs are for organ histopathology.
			Cardio	150				
			Gastro	150				
			Hemato	5 F	75 F (decrease platelets, erythrocyte counts, and hematocrit)			
			Musc/skel	150				
			Hepatic	5 M	75 M (increased serum ALT activity)			
			Renal	150				
			Endocr	5	75 (decrease serum T4)			
			Ocular	75		150 (retinal degeneration, cataracts)		
			Bd Wt	5 F	75 F (11% reduced weight gain)	150 F (43% reduced weight gain)		
			Metab	150				

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Table 3-2 Levels of Significant Exposure to 2,4-Dichlorophenoxyacetic Acid - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
102	Rat (Osborne-Mendel)	2 yr ad lib (F)	Resp	92.5			Hansen et al. 1971 2,4-dichlorophenoxyacetic acid	
			Cardio	92.5				
			Gastro	92.5				
			Musc/skel	92.5				
			Hepatic	92.5				
			Renal	92.5				
			Endocr	92.5				
			Bd Wt	92.5				

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Table 3-2 Levels of Significant Exposure to 2,4-Dichlorophenoxyacetic Acid - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
103	Mouse (B6C3F1)	2 yr ad lib (F)	Resp	300 F			Charles et al. 1996b 2,4-dichlorophenoxyacetic acid	NOAELs are for organ histopathology.
			Cardio	300 F				
			Gastro	300 F				
			Hemato	300 F				
			Musc/skel	300 F				
			Hepatic	300 F				
			Renal	5 M	62.5 M[b] (degeneration/ regeneration proximal tubule)			
					150 F (degeneration/ regeneration proximal tubule)			
			Endocr	300 F				
			Ocular	300 F				
			Bd Wt	300 F				

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Table 3-2 Levels of Significant Exposure to 2,4-Dichlorophenoxyacetic Acid - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
104	Mouse (B6C3F1)	104 wk ad lib (F)	Resp	45			EPA 1987a 2,4-dichlorophenoxyacetic acid	
			Cardio	45				
			Gastro	45				
			Hemato	45				
			Musc/skel	45				
			Hepatic	45				
			Renal	1 M	15 M (reduced cytoplasmic vacuoles)			
			Endocr	1 M 45 F	15 M[b] (increased absolute and relative adrenals weight)			
			Dermal	45				
			Ocular	45				
			Bd Wt	45				

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Table 3-2 Levels of Significant Exposure to 2,4-Dichlorophenoxyacetic Acid - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
105	Dog (Beagle)	2 yr ad lib (F)	Resp	10			Hansen et al. 1971 2,4-dichlorophenoxyacetic acid	NOAELs are for organ histopathology.
			Cardio	10				
			Gastro	10				
			Musc/skel	10				
			Hepatic	10				
			Renal	10				
			Endocr	10				
Immuno/ Lymphoret								
106	Rat (Fischer- 344)	2 yr ad lib (F)		150			Charles et al. 1996b 2,4-dichlorophenoxyacetic acid	NOAEL is for histopathology of lymphoreticular tissues.
107	Rat (Osborne-Mendel)	2 yr ad lib (F)		92.5			Hansen et al. 1971 2,4-dichlorophenoxyacetic acid	NOAEL is for histopathology of the spleen.
108	Mouse (B6C3F1)	2 yr ad lib (F)		300 F			Charles et al. 1996b 2,4-dichlorophenoxyacetic acid	NOAEL is for histopathology of lymphoreticular organs.
109	Mouse (B6C3F1)	104 wk ad lib (F)		45			EPA 1987a 2,4-dichlorophenoxyacetic acid	NOAEL is for histopathology of lymphoreticular tissues.

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Table 3-2 Levels of Significant Exposure to 2,4-Dichlorophenoxyacetic Acid - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
110	Dog (Beagle)	2 yr ad lib (F)		10			Hansen et al. 1971 2,4-dichlorophenoxyacetic acid	NOAELs are for histopathology of spleen and lymph nodes.
Neurological								
111	Rat (Fischer- 344)	2 yr ad lib (F)		150			Charles et al. 1996b 2,4-dichlorophenoxyacetic acid	NOAEL is for histopathology of central and peripheral neural tissues.
112	Mouse (B6C3F1)	2 yr ad lib (F)		300 F			Charles et al. 1996b 2,4-dichlorophenoxyacetic acid	NOAEL is for central and peripheral neural tissue histopathology.
113	Mouse (B6C3F1)	104 wk ad lib (F)		45			EPA 1987a 2,4-dichlorophenoxyacetic acid	NOAEL is for histopathology of central and peripheral nervous tissues.
114	Dog (Beagle)	2 yr ad lib (F)		10			Hansen et al. 1971 2,4-dichlorophenoxyacetic acid	NOAELs are for histopathology of brain and spinal cord.
Reproductive								
115	Rat (Fischer- 344)	2 yr ad lib (F)		150			Charles et al. 1996b 2,4-dichlorophenoxyacetic acid	NOAEL is for histopathology of reproductive organs of males and females.

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Table 3-2 Levels of Significant Exposure to 2,4-Dichlorophenoxyacetic Acid - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
116	Rat (Osborne-Mendel)	2 yr ad lib (F)		92.5			Hansen et al. 1971 2,4-dichlorophenoxyacetic acid	NOAEL is for histopathology of the reproductive organs.
117	Mouse (B6C3F1)	2 yr ad lib (F)		125 M 300 F			Charles et al. 1996b 2,4-dichlorophenoxyacetic acid	NOAEL is for histopathology of reproductive organs.
118	Mouse (B6C3F1)	104 wk ad lib (F)		45			EPA 1987a 2,4-dichlorophenoxyacetic acid	NOAEL is for histopathology of reproductive organs.
119	Dog (Beagle)	2 yr ad lib (F)		10			Hansen et al. 1971 2,4-dichlorophenoxyacetic acid	NOAELs are for histopathology of reproductive organs.

a The number corresponds to entries in Figure 3-2.

b Differences in levels of health effects between male and female are not indicated in Figure 3-2. Where such differences exist, only the levels of effect for the most sensitive gender are presented.

c Used to derive an intermediate-duration oral (MRL) of 0.009 mg/kg/day for 2,4-D. Using benchmark-dose modeling, a BMDRD05 of 1.27 mg 2,4-D/kg/day and a BMDLRD05 of 0.93 mg 2,4-D/kg/day, respectively, were calculated for reduced rat offspring body weight from the selected model (Exponential model 4). The BMDLRD05 was divided by an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability) to derive the MRL of 0.009 mg/kg/day. The intermediate-duration oral MRL was also adopted as acute-duration oral MRL for 2,4-D.

ad lib = ad libitum; ALT = alanine aminostransferase; Bd Wt = body weight; BUN = blood urea nitrogen; (C) = capsule; Cardio = cardiovascular; CNS = central nervous system; d = day(s); Endocr = endocrine; (F) = feed; F = Female; (G) = gavage; Gastro = gastrointestinal; Gd = gestational day; gen = generation; Gn pig = guinea pig; (GO) = gavage in oil; (GW) = gavage in water; Hemato = hematological; Immuno/Lymphoret = immunological/lymphoreticular; LD50 = lethal dose, 50% kill; LOAEL = lowest-observed-adverse-effect level; M = male; Metab = metabolism; mo = month(s); Musc/skel = musculoskeletal; NOAEL = no-observed-adverse-effect level; NS = not specified; Pnd = post-natal day; Ppd = post-parturition day; Resp = respiratory; T3 = triiodothyronine; T4 = thyroxine; TSH = thyroid-stimulating hormone; x = time(s); (W) = drinking water; wk = week(s); yr = year(s)

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Figure 3-2 Levels of Significant Exposure to 2,4-Dichlorophenoxyacetic Acid - Oral

Acute (≤14 days)

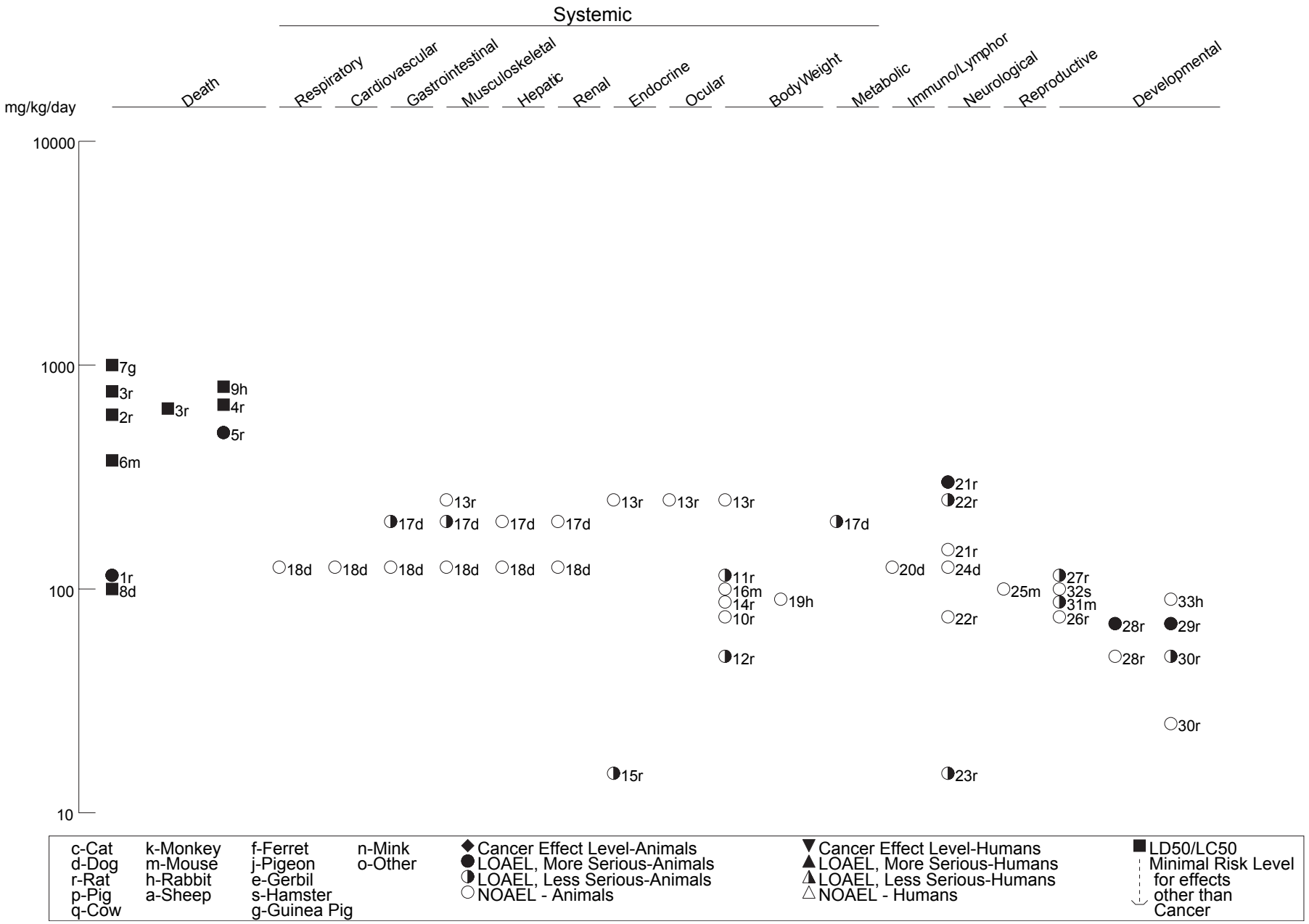

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Figure 3-2 Levels of Significant Exposure to 2,4-Dichlorophenoxyacetic Acid - Oral *(Continued)*

Intermediate (15-364 days)

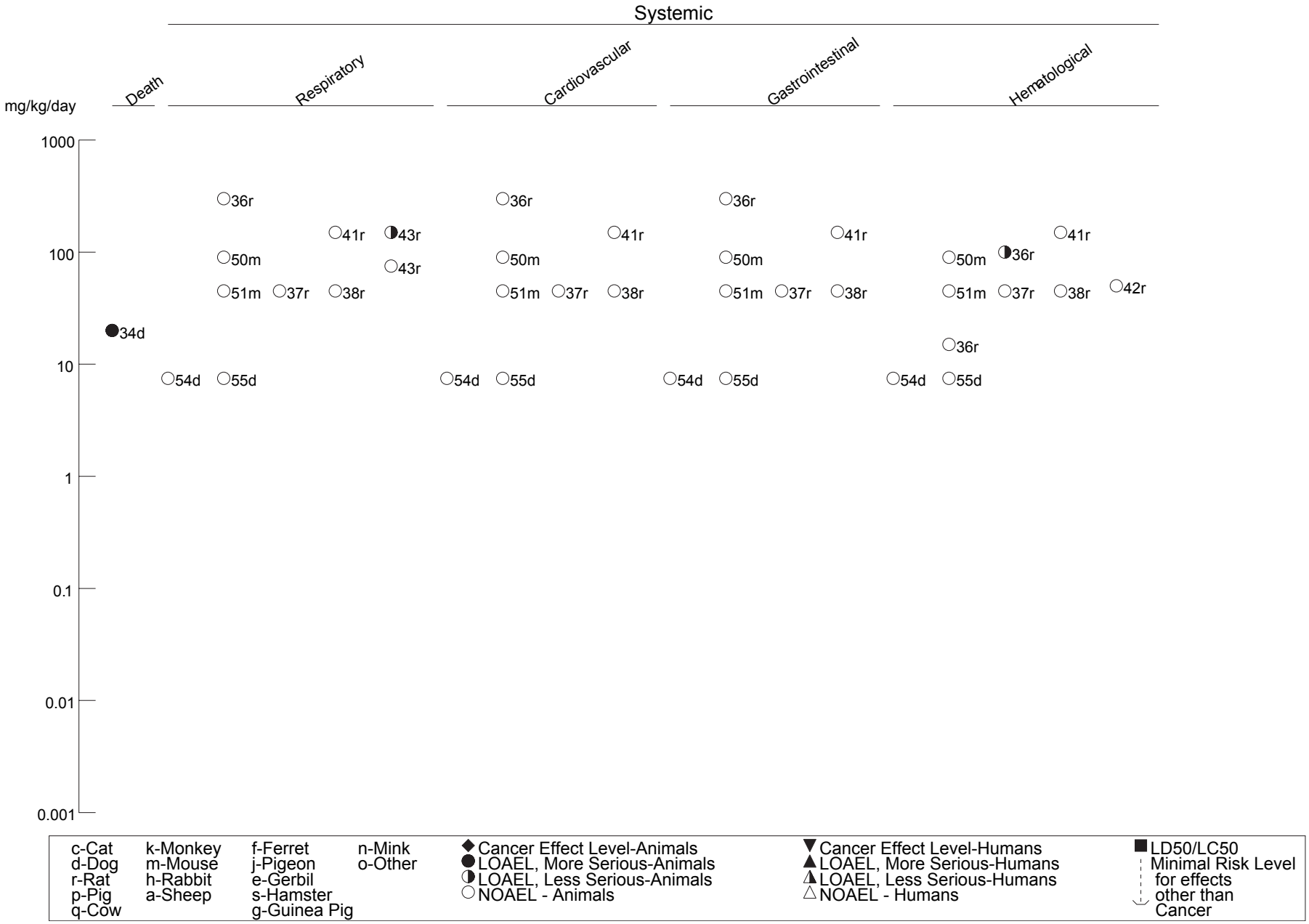

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Figure 3-2 Levels of Significant Exposure to 2,4-Dichlorophenoxyacetic Acid - Oral *(Continued)*

Intermediate (15-364 days)

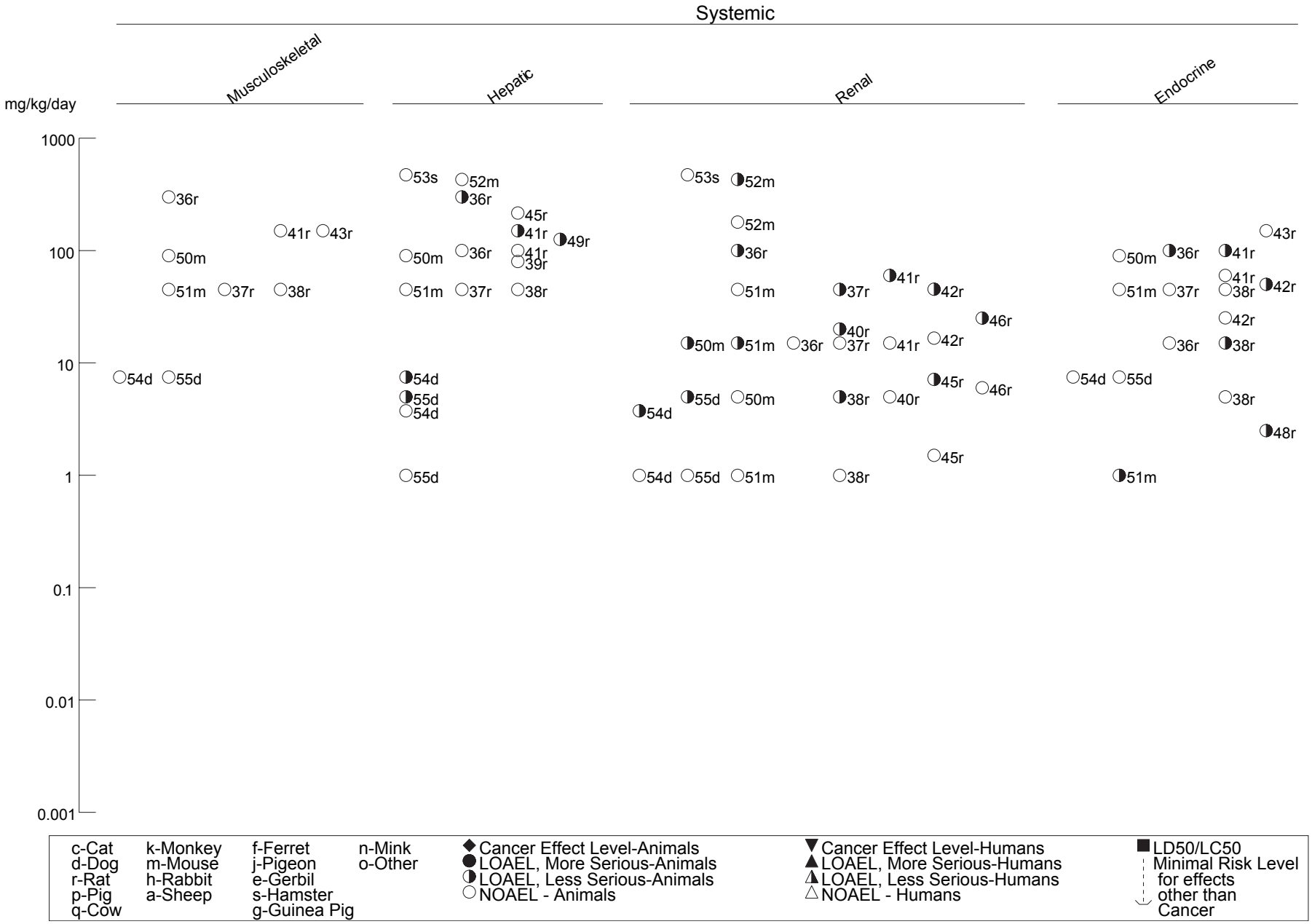

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Figure 3-2 Levels of Significant Exposure to 2,4-Dichlorophenoxyacetic Acid - Oral *(Continued)*

Intermediate (15-364 days)

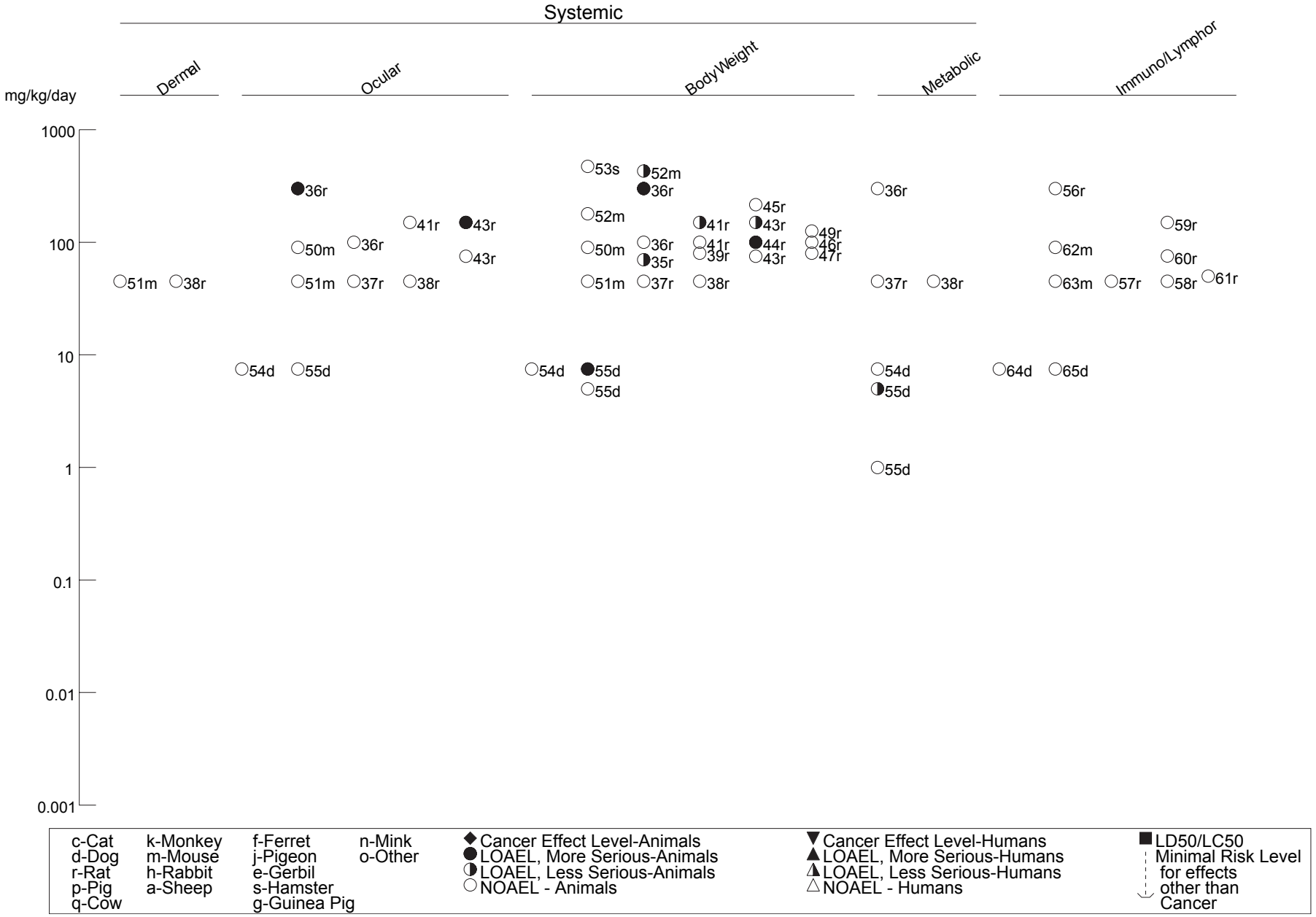

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Figure 3-2 Levels of Significant Exposure to 2,4-Dichlorophenoxyacetic Acid - Oral *(Continued)*

Intermediate (15-364 days)

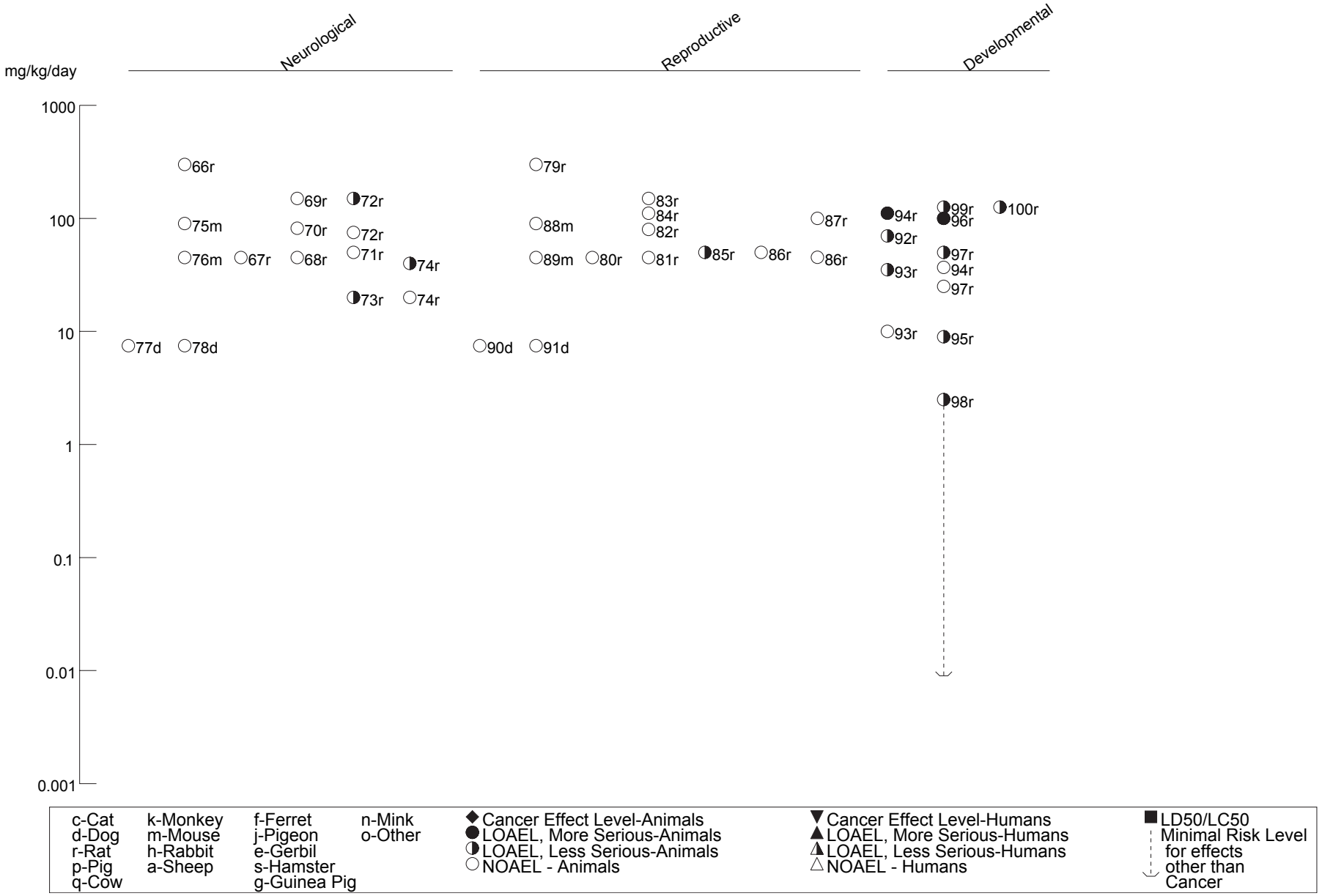

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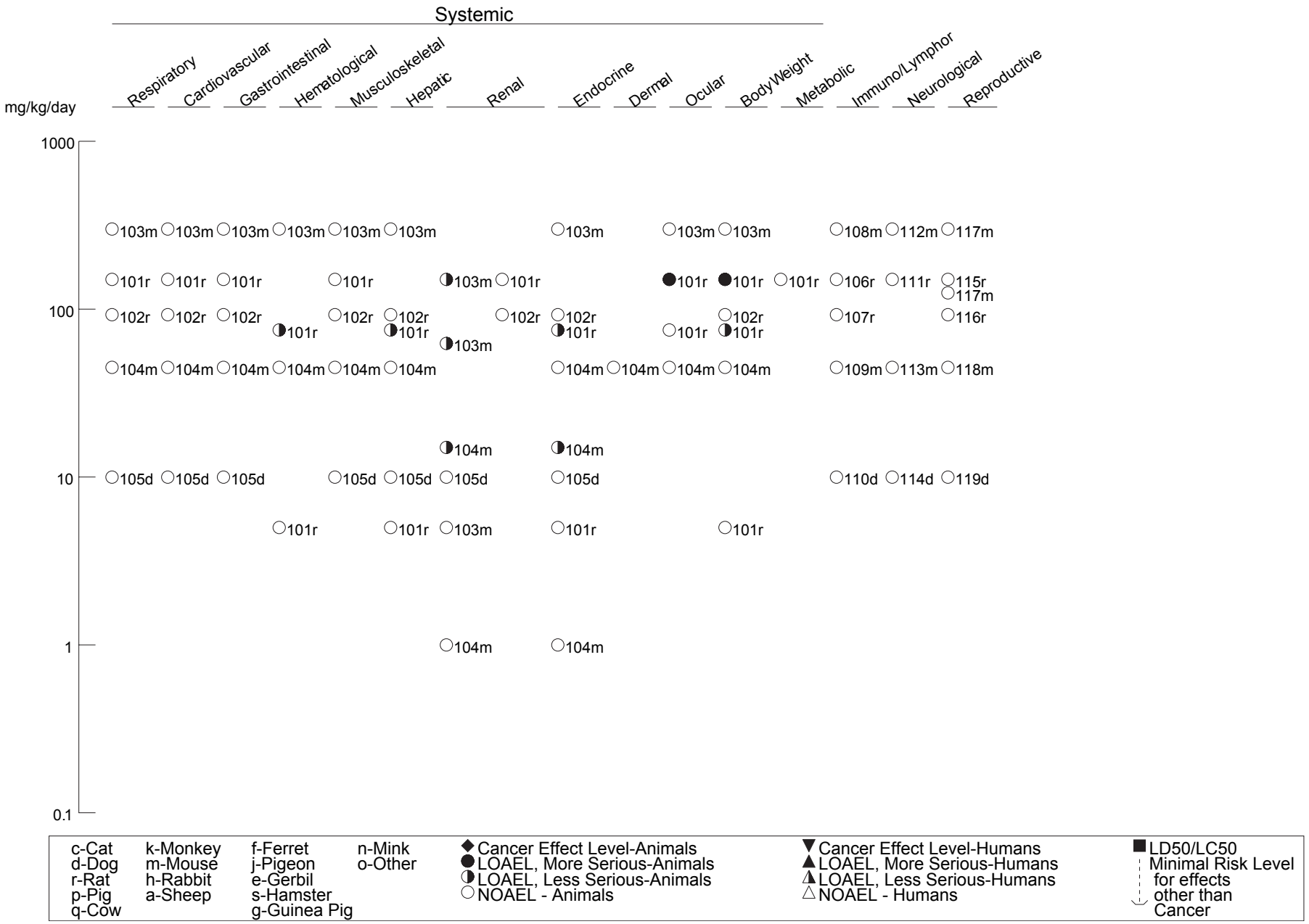
Figure 3-2 Levels of Significant Exposure to 2,4-Dichlorophenoxyacetic Acid - Oral *(Continued)*

Chronic (≥365 days)



Similar results were reported in chronic-duration studies in rats exposed to up to 150 mg 2,4-D/kg/day (Charles et al. 1996b; Hansen et al. 1971), mice exposed to ≤300 mg 2,4-D/kg/day (Charles et al. 1996b; EPA 1987a), and dogs exposed to 10 mg 2,4-D/kg/day (Hansen et al. 1971).

The only effect attributed to exposure to 2,4-D was the finding of pale foci in the lungs from four out of five female rats exposed to 150 mg 2,4-D/kg/day for 52 weeks; no alterations were seen at 75 mg/kg/day (Mattsson et al. 1997).

No definite conclusions can be drawn regarding respiratory effects after oral exposure to 2,4-D based solely on morphological evaluations of the respiratory tract in animal studies; it does not seem that the lungs are a particularly sensitive organ for ingested 2,4-D in animals at doses that do not induce overt effects.

Cardiovascular Effects. Tachycardia was reported in two of the four cases of intoxication with an herbicide containing 2,4-D reported by Durakovic et al. (1992). One person had ingested approximately 100 mL of a 40% solution of 2,4-D (40 g); the other individual had ingested 400 mL of a 40% solution of a commercial herbicide (140 g). Tachycardia was also reported in the fatal case reported by Keller et al. (1994). Normal blood pressure and electrocardiogram (except for a sinus tachycardia) were observed in a subject who ingested approximately 110 mg 2,4-D/kg from a commercial herbicide product (Berwick 1970).

Information regarding cardiovascular effects in animals is limited to results of morphological examination of the heart. No alterations were reported in the heart from dogs following administration of a single dose of ≤125 mg 2,4-D/kg (Steiss et al. 1987). In intermediate-duration studies, no effects were reported in rats exposed to ≤300 mg 2,4-D/kg/day (Charles et al. 1996a; EPA 1984, 1985; Gorzinski et al. 1987), mice exposed to ≤90 mg 2,4-D/kg/day (EPA 1984, 1987a), or dogs exposed to 7.5 mg 2,4-D/kg/day (Charles et al. 1996c).

Similar negative results were reported in chronic-duration studies in rats exposed to ≤150 mg 2,4-D/kg/day (Charles et al. 1996b; Hansen et al. 1971), mice exposed to ≤300 mg 2,4-D/kg/day (Charles et al. 1996b; EPA 1987a), and dogs exposed to ≤10 mg 2,4-D/kg/day (Hansen et al. 1971).

Based on the information available, it does not appear that the cardiovascular system is a sensitive target for 2,4-D.

Gastrointestinal Effects. Nausea and vomiting has been reported following ingestion of products containing 2,4-D (Berwick 1970; Keller et al. 1994; Nielsen et al. 1965). Abdominal sonography and gastroscopy performed in the case reported by Keller et al. (1994) revealed massive damage of the esophagus and accumulation of blood in the stomach. Furthermore, the stomach mucosa indicated signs of massive hemorrhage and mild necrosis. Autopsy performed on the lethal case studied by Dudley and Thapar 1972) showed markedly hyperemic stomach, duodenum, and proximal jejunum. Light microscopy of the esophagus, stomach, and duodenum showed severe congestion of vessels throughout the mucosa and submucosa. This limited information suggests that bolus ingestion of commercial products containing 2,4-D can produce severe irritation to mucosal membranes.

For the most part, information regarding gastrointestinal effects in animals is limited to results of morphological examination of the gastrointestinal tract. No alterations were reported in the gastrointestinal tract from dogs following administration of a single dose of ≤125 mg 2,4-D/kg in a gelatin capsule (Steiss et al. 1987). Another acute-duration study reported that vomiting was observed in two out of six female dogs given a dose of 200 mg 2,4-D/kg in a gelatin capsule, and all six dogs had diarrhea (Dickow et al. 2000).

No significant morphological alterations in the gastrointestinal tract were reported in intermediate-duration studies in rats exposed to ≤300 mg 2,4-D/kg/day (Charles et al. 1996a; EPA 1984, 1985; Gorzinski et al. 1987), mice exposed to ≤90 mg 2,4-D/kg/day (EPA 1984, 1987a), or dogs exposed to 7.5 mg 2,4-D/kg/day (Charles et al. 1996c).

Similar results were reported in chronic-duration studies in rats exposed to ≤150 mg 2,4-D/kg/day (Charles et al. 1996b; Hansen et al. 1971), mice exposed to ≤300 mg 2,4-D/kg/day (Charles et al. 1996b; EPA 1987a), and dogs exposed to 10 mg 2,4-D/kg/day (Hansen et al. 1971).

The data in animals suggest that relatively high doses of 2,4-D are unlikely to cause gastrointestinal irritation if 2,4-D is mixed in the food.

Hematological Effects. The only information available in humans following oral exposure to 2,4-D is that apparent leukocytosis occurred in two of the four cases of intoxication with products containing 2,4-D described by Durakovic et al. (1992). No other relevant information was located.

No information was located regarding hematological effects in animals in acute-duration studies. Intermediate- and chronic-duration studies reported some statistically significant differences in hematological parameters between treated and control rats. Significantly decreased platelet counts were reported in male and female rats exposed to ≥100 mg 2,4-D/kg/day for 13 weeks; the NOAEL was 15 mg/kg/day (Charles et al. 1996a). Hemoglobin and red blood cell counts were also decreased in male and female rats exposed to 300 mg 2,4-D/kg/day for 13 weeks (Charles et al. 1996a). EPA (1984) reported that male rats showed significant decreases in hemoglobin in rats exposed to ≥1 mg 2,4-D/kg/day for 13 weeks, but the values were well within the normal range. Another 13-week study reported a NOAEL of 150 mg/kg/day (highest dose tested) for hematological effects, but platelet counts were not determined (Gorzinski et al. 1987). No significant hematological alterations were reported in mice exposed to ≤90 mg 2,4-D/kg/day for 13 weeks (EPA 1984) or ≤45 mg/kg/day for 52 weeks (EPA 1987a), or in dogs exposed to ≤7.5 mg 2,4-D/kg/day for 52 weeks (Charles et al. 1996c).

A chronic-duration study reported that exposure of rats to ≥75 mg 2,4-D/kg/day for 2 years induced significant decreases in platelet counts, erythrocyte counts, and hematocrit in females; the NOAEL was 5 mg/kg/day (Charles et al. 1996b). In contrast, no significant hematological alterations were reported in mice exposed to ≤300 mg 2,4-D/kg/day for 2 years (Charles et al. 1996b), suggesting that mice are less susceptible than rats to 2,4-D-induced hematological effects.

Musculoskeletal Effects. Spontaneous fibrillary twitching in the muscles of the upper extremities was reported in a subject 24 hours after ingestion of approximately 110 mg 2,4-D/kg (Berwick 1970). The only additional relevant information is that an autopsy of a man who died after consuming an unknown amount of 2,4-D did not reveal abnormalities in the musculoskeletal system (Dudley and Thapar 1972).

Limited information is available from acute-duration studies. A single gavage dose of 250 mg 2,4-D/kg (highest dose tested) did not induce gross or microscopic alterations in skeletal muscle from rats (Mattsson et al. 1997). However, 200 mg 2,4-D/kg administered in a gelatin capsule to six female dogs induced prolonged insertional electrical activity (electromyography [EMG]) in all dogs and fibrillation potentials in one dog, indicating possible muscle pathology (Dickow et al. 2000). Mean total and unbound concentrations of 2,4-D in plasma at the time of the electromyographic evaluation were 511 and 129 mg/L, respectively. Transient myotonia was reported in female dogs given a single dose of ≥50 mg 2,4-D/kg; however, no histological alterations were reported in skeletal muscles examined 28 days after administration of a single dose of ≤125 mg 2,4-D/kg (Steiss et al. 1987).

Intermediate-duration studies provide information on skeletal muscle and bone morphology after oral exposure to 2,4-D. No significant effects were reported in rats exposed to ≤300 mg 2,4-D/kg/day for intermediate durations (Charles et al. 1996a; EPA 1984, 1985; Gorzinski et al. 1997), mice exposed to ≤90 mg 2,4-D/kg/day (EPA 1984, 1987a), or dogs exposed to ≤7.5 mg 2,4-D/kg/day (Charles et al. 1996c).

Similar results were reported in chronic-duration studies in rats exposed to ≤150 mg 2,4-D/kg/day (Charles et al. 1996b; Hansen et al. 1971), mice exposed to ≤300 mg 2,4-D/kg/day (Charles et al. 1996b; EPA 1987a), and dogs exposed to ≤10 mg 2,4-D/kg/day (Hansen et al. 1971).

Although animals tested in the long-term oral studies did not exhibit clinical signs (i.e., altered posture or gait) that could suggest skeletal muscle alterations, it would be helpful to have information on muscle physiology following prolonged exposure to 2,4-D.

Hepatic Effects. Liver congestion was observed at autopsy in the fatal intoxication case reported by Nielsen et al. (1965). Gross necropsy of the liver in the lethal case reported by Dudley and Thapar (1972) showed hyperemic liver; microscopic examination showed diffuse acute necrosis. Significant increases in liver enzymes were reported in a man who ingested approximately 110 mg 2,4-D/kg from a commercial herbicide product and survived (Berwick 1970). No general conclusions regarding hepatic effects of ingested 2,4-D in humans can be made based on only these two case reports.

Limited data from acute-duration studies in animals showed that in dogs, a single dose of 125 mg 2,4-D/kg in a gelatin capsule did not induce histological alterations in the liver (Steiss et al. 1987) and a dose of 200 mg/kg did not significantly alter clinical chemistry parameters used to assess liver function (Dickow et al. 2000).

More information is available regarding hepatic effects in animals in longer-term studies, especially intermediate-duration studies. Results in rats show apparent inconsistencies between studies. In general, results suggest species differences in sensitivity, with dogs being more sensitive than rodents.

Increased absolute liver weight, liver histopathology, increased serum transaminases, and oxidative stress were reported in Wistar rats exposed to 126 mg 2,4-D/kg/day (only dose tested, administered in drinking water) on GDs 14–21 and on postnatal days (PNDs) 0–14 (Troudi et al. 2012a). However, dietary doses

of approximately 215 mg 2,4-D/kg/day (highest dose tested) did not cause histological alterations in the liver from Sprague-Dawley rats in a 13-week study (Ozaki et al. 2001). In three additional 13-week dietary studies in F-344 rats, doses of ≥150 mg 2,4-D/kg/day induced histological alterations in the liver and the NOAEL was 100 mg/kg/day (Charles et al. 1996a; EPA 1984; Gorzinski et al. 1987). A 2-generation reproductive study reported a NOAEL of 80 mg 2,4-D/kg/day for liver histopathology in the parental and F1 generations (EPA 1986).

In mice, exposures to ≤429 mg 2,4-D/kg/day for 13 weeks (EPA 1984; Ozaki et al. 2001) or ≤45 mg/kg/day for 52 weeks (EPA 1987a) did not induce histological alterations in the liver. Similarly, hamsters exposed via the diet to ≤474 mg 2,4-D/kg/day for 13 weeks did not show treatment-related lesions in the liver (Ozaki et al. 2001). In dogs, however, doses of ≤7.5 mg 2,4-D/kg/day for 13 weeks induced what was described as perivascular active inflammation in the liver; the NOAEL was 3.75 mg/kg/day (Charles et al. 1996c).

Chronic-duration studies in rats showed that increasing the duration of exposure from 13 weeks to 2 years did not result in increased incidence or severity of the liver alterations reported at 150 mg 2,4-D/kg/day in the 13-week study (Gorzinski et al. 1987). Rats exposed for 2 years to 150 mg 2,4-D/kg/day showed only increased incidence of "minimal panlobular tinctorial properties," exposure to 75 mg/kg/day increased serum ALT activity, and the NOAEL was 5 mg/kg/day (Charles et al. 1996b). In mice, exposure for 2 years to ≤300 mg 2,4-D/kg/day did not induce liver histopathology (Charles et al. 1996b) and the same was reported in dogs exposed for 2 years up to 10 mg 2,4-D/kg/day (Hansen et al. 1971).

Results from animal studies suggest that minimal liver pathology occurs in animals at exposure levels considerably higher than would be encountered by humans due to environmental exposures (in the µg 2,4-D/kg/day range).

Renal Effects. Renal congestion, but no degenerative changes in the kidneys, was observed in a fatal case reported by Nielsen et al. (1965). Acute kidney failure preceding death was reported in a case described by Keller et al. (1994) and in a case that survived intoxication as described by Durakovic et al. (1992). In a fatal case of intoxication with 2,4-D reported by Dudley and Thapar (1972), autopsy revealed a hyperemic renal medulla. Microscopic examination of the kidneys showed mildly active chronic pyelonephritis, moderate arteriolar sclerosis, congestion of the capillaries of the medulla, and dilated collecting tubules.

Only two acute-duration oral studies in dogs examined renal end points. No significant histopathological alterations were reported in the kidneys from dogs administered a single dose of 125 mg 2,4-D/kg (highest dose tested) (Steiss et al. 1987). A single dose of 200 mg 2,4-D/kg (only dose tested) did not significantly affect clinical chemistry parameters normally used to monitor kidney function; no histopathological assessment was conducted in this study (Dickow et al. 2000).

Alterations in the kidneys have been reported in intermediate-duration oral studies in rats, but there are some apparent inconsistencies between studies. The lowest LOAEL was approximately 7.1 mg 2,4-D/kg/day reported in a 13-week study; the NOAEL was 1.5 mg/kg/day (Ozaki et al. 2001). The alterations were diagnosed as simple hyperplasia. The lesion was located in the outer stripe of the outer medulla and consisted of a few scattered foci of tubules with prominent basophilia due to high nuclear density and decreased cytoplasmic volume of the epithelial cells. This was not observed in hamsters exposed to ≤474 mg 2,4-D/kg/day for 13 weeks (Ozaki et al. 2001). Other 13-week or shorter duration studies in rats reported LOAELs for histopathological alterations in the kidneys at doses in the range of 40–60 mg 2,4-D/kg/day (EPA 1984; Gorzinski et al. 1987; Marty et al. 2013; Saghir et al. 2013). NOAELs ranged from 6 to 15 mg 2,4-D/kg/day. Renal clearance of 2,4-D is saturated at different levels in adult female (14–27 mg/kg/day) and adult male (approximately 63 mg/kg/day) rats (Saghir et al. 2013). However, Charles et al. (1996a) reported kidney histopathology in male and female rats only at 300 mg 2,4-D/kg/day for 13 weeks, but not after exposure to 100 mg/kg/day, and a 2-generation study reported a LOAEL of 20 mg/kg for kidney histopathology in rats (EPA 1987b). A 52-week study reported increased tubular cell brown pigments in male and female rats exposed to 15 mg 2,4-D/kg/day; females also showed fine vacuolization of the cytoplasm in the renal cortex at 15 mg/kg/day; the NOAEL was 5 mg/kg/day (EPA 1985). Chronic-duration studies did not report kidney lesions in rats exposed to ≤150 mg 2,4-D/kg/day for 2 years (Charles et al. 1996b; Hansen et al. 1971).

The picture is not clear in mice either. Changes described as increased homogeneity and altered tinctorial properties of the cytoplasm and decreased intracellular/intraluminal vacuolization in the cortex were reported in male mice exposed to 15 mg 2,4-D/kg/day for 13 or 52 weeks; NOAELs were 1–5 mg/kg/day (EPA 1984, 1987a). However, in another 13-week study, kidney lesions were reported in male mice after exposure to approximately 430 mg 2,4-D/kg/day, but not in mice exposed to approximately 179 mg/kg/day (Ozaki et al. 2001). Two-year exposures of mice to ≥15 mg 2,4-D/kg/day also resulted in kidney alterations; NOAELs were in the 1–5 mg/kg/day range (Charles et al. 1996b; EPA 1987a).

No histological alterations were seen in the kidneys from dogs exposed to ≤7.5 mg 2,4-D for intermediate durations, but there was some indication of altered kidney function assessed as increased BUN and serum creatinine (Charles et al. 1996c). Hansen et al. (1971) did not find morphological alterations in the kidneys from dogs exposed to ≤10 mg 2,4-D/kg/day for 2 years; however, clinical chemistry tests were not conducted in this study, so kidney function was not addressed.

Kidney effects were observed in all of the animal species tested, but with the wide range of results available, it is difficult to make generalizations.

Endocrine Effects. The only relevant information regarding endocrine effects in humans following oral exposure to 2,4-D is that acute congestion was seen in the adrenals in the lethal case reported by Nielsen et al. (1965) and that the endocrine system appeared normal at autopsy in the case reported by Dudley and Thapar (1972).

Studies in animals provide information on gross and microscopic morphology of endocrine glands following long-term oral exposure to 2,4-D. Results from some studies showed alterations in serum levels of thyroid hormones and prolactin.

Serum levels of prolactin were significantly decreased in rats administered doses ≥2.5 mg 2,4-D/kg/day on postpartum days 1–16 (Stürtz et al. 2008, 2010). This effect was attributed in part to decreased levels of serotonin and increased levels of dopamine in the arcuate nucleus of the brain (Stürtz et al. 2008, 2010).

Alterations in thyroid hormone levels have been reported in rats in long-term studies. For example, serum thyroxine (T4) and triiodothyronine (T3) were significantly reduced in female rats following exposure to 100 mg 2,4-D/kg/day for 13 weeks; the NOAEL was 15 mg/kg/day (Charles et al. 1996a). Decreased serum T4 was also reported in females exposed to 100 mg 2,4-D/kg/day in another 13-week study (Gorzinski et al. 1987). In contrast, T4 was elevated in male rats at 300 mg 2,4-D/kg/day (Charles et a; 1996a) and EPA (1984) reported that serum T4 was increased in male rats exposed to 5 or 15 mg 2,4-D/kg/day for 13 weeks, but no significant change was seen in rats exposed to 45 mg 2,4-D/kg/day. Also, EPA (1985) reported that female rats exposed to ≥15 mg 2,4-D/kg/day for 27 weeks had significantly increased serum T4, but no increase was evident after 52 weeks of exposure and no alterations were seen in males exposed to ≤45 mg 2,4-D/kg/day at either time point. In none of these studies were there histological alterations in the thyroid. Pregnant rats exposed to approximately 50 mg

2,4-D/kg/day from pre-breeding through GD 17 had nonsignificant decreased serum T3 and T4 and increased TSH on GD 17 (Marty et al. 2013). The investigators also noted that 3 out of 12 females had histological alterations consisting of smaller thyroid follicles with small vacuoles in the colloid, which suggested colloid resorption. Because there were no adverse pathological alterations and thyroid changes in dams exposed similarly and examined on lactation day 21, the investigators suggested that the changes were transient, and therefore, were considered adaptive, yet exposure related. Dose-related decreases in serum T4 were also reported in male and female rats exposed to ≥75 mg 2,4-D/kg/day for 2 years; the NOAEL was 5 mg/kg/day (Charles et al. 1996b). There were no histopathological alterations in either sex exposed to ≤150 mg 2,4-D/kg/day.

Adrenal cortex hypertrophy was reported in female rats exposed to 100 mg 2,4-D/kg/day for 13 weeks (Charles et al. 1996a). Male mice exposed to ≥1 mg 2,4-D/kg day for 52 weeks showed significant decreases in absolute and relative adrenals weight, but exposure to ≥15 mg 2,4-D/kg/day for 104 weeks resulted in significant increases in absolute and relative adrenals weight (EPA 1987a). In the absence of histopathology, the toxicological significance of these changes in adrenal weight is unknown.

In summary, the fact that relatively low doses of 2,4-D reduced serum levels of prolactin in postpartum rats is significant in that it resulted in reduced offspring body weight. In humans, prolactin is critical for the establishment of lactation, for milk production, and for an adequate milk macronutrient content (Ostrom 1990). Alterations in thyroid hormones in rats unaccompanied by pathological changes in the thyroid gland occur at exposure levels unlikely to be found in the environment. Further generational and neurological studies would be beneficial to add weight of the evidence to findings.

Dermal Effects. No information was located regarding dermal effects in humans following oral exposure to 2,4-D.

The only information regarding dermal effects in animals following oral exposure to 2,4-D is that no histological alterations were seen in the skin of rats and mice exposed to ≤45 mg 2,4-D/kg/day for 52 weeks (EPA 1985, 1987a) or mice exposed to ≤45 mg/kg/day for 2 years (EPA 1987a).

Ocular Effects. No information was located regarding ocular effects in humans following oral exposure to 2,4-D.

Ocular effects were reported in rats in intermediate- and chronic-duration studies; no ocular effects were reported in other animal species tested. Acute administration of a single doses of ≤250 mg 2,4-D/kg to rats did not induce histological alterations in the eye, but 150 mg/kg/day given chronically for 52 weeks induced bilateral retinal degeneration in five out of five females; no treatment-related lesions were seen at 75 mg/kg/day (Mattsson et al. 1997). The degeneration was characterized by a complete loss of the rod and cone layer and the outer and inner nuclear layers. Thirteen-week intermediate-duration studies established a NOAEL of 150 mg/kg/day for ocular lesions in rats (Gorzinski et al. 1987), but exposure to 300 mg 2,4-D/kg/day induced retinal degeneration and cataract formation in female rats (Charles et al. 1996a).

Chronic-duration studies confirmed the existence of an exposure-duration factor evident in intermediate-duration studies as exposure to 150 mg 2,4-D/kg/day for 2 years caused constriction of blood vessels and hyperreflectivity of the fundus in male rats and lens opacity in female rats (Charles et al. 1996b). Microscopically, both sexes showed retinal degeneration and cataracts; the incidence of ocular lesions was not significantly elevated in rats exposed to ≤75 mg 2,4-D/kg/day.

Though rat studies indicate that ocular lesions/degeneration is possible from 2,4-D exposure, the significance of this finding to humans is unknown. It should be noted also that the lesions appear to occur at exposure levels much higher than from exposure to environmental levels of 2,4-D.

Body Weight Effects. No information was located regarding body weight effects in humans following oral exposure to 2,4-D.

Many animal studies monitored body weight, but making generalizations is difficult due to apparent inconsistencies between studies. Apparent inconsistencies may be due to testing animals of different ages (i.e., adults versus growing animals) or pregnant females, which could be more susceptible than nonpregnant females. Studies do not always provide data on food consumption. Even if they do, reduced food consumption in dietary studies may be due, in part, to poor palatability.

In rats administered a single gavage dose of 250 mg 2,4-D/kg, body weight was not affected over the next 15 days (Mattsson et al. 1997). Dosing of pregnant Wistar rats with ≥50 mg 2,4-D/kg/day by gavage on GDs 6–15 resulted in significant dose-related weight loss during pregnancy (Fofana et al. 2000), but dosing pregnant F-344 rats by gavage with ≤75 mg 2,4-D/kg/day or pregnant Sprague-Dawley rats with ≤87.5 mg 2,4-D/kg/day on GDs 6–15 did not significantly affect weight gain during treatment (Charles et

al. 2001; Schwetz et al. 1971), suggesting that Wistar rats are more susceptible than F-344 rats. However, dosing pregnant Sprague-Dawley rats with 115 mg 2,4-D/kg/day on GDs 6–15 resulted in reduced weight gain during treatment (Chernoff et al. 1990). No effects were reported in pregnant rabbits dosed by gavage with 90 mg 2,4-D/kg/day on GDs 6–18 (Charles et al. 2001). Body weight was not significantly affected in mice dosed with 100 mg 2,4-D/kg via drinking water for 10 days (Dinamarca et al. 2007).

Intermediate-duration studies in rats provide a less-than-clear picture. Three studies reported a NOAEL of 100 mg 2,4-D/kg/day (Charles et al. 1996a; Gorzinski et al. 1987; Saghir et al. 2013). Doses ≥150 mg 2,4-D/kg/day significantly decreased body weight gain (Charles et al. 1996a; Gorzinski et al. 1987; Mattsson et al. 1997). A 5-week study in rats reported a NOAEL of 80 mg 2,4-D/kg/day (Squibb et al. 1983), whereas a 13-week study reported no significant effects on body weight in rats dosed with 215 mg 2,4-D/kg/day (Ozaki et al. 2001). A study in pregnant rats reported a LOAEL of 100 mg 2,4-D/kg/day for significantly reduced weight gain during pregnancy (Mazhar et al.2014), while another reported a NOAEL (5% difference between treated and controls) of 126 mg/kg/day (Troudi et al. 2012a). Male offspring from rats exposed to 70 mg 2,4-D/kg/day (only dose tested) during gestation and lactation and then directly showed an 11% reduction in body weight relative to controls at 90 days of age (Bortolozzi et al. 1999).

The highest NOAEL for body weight effects in intermediate-duration studies in mice was 178.9 mg 2,4-D/kg/day; the LOAEL was 429.4 mg/kg/day (Ozaki et al. 2001). Dogs exposed to 7.5 mg 2,4-D/kg/day for 52 weeks showed a 64% reduction in weight gain relative to controls; the NOAEL was 5 mg/kg/day (Charles et al. 1996c). Body weight was not significantly affected in hamsters exposed to 474 mg 2,4-D/kg/day for 3 months (Ozaki et al. 2001).

Chronic-duration studies reported NOAEL and LOAEL values of 5 and 75 mg 2,4-D/kg/day, respectively, for body weight in rats (Charles et al. 1996b) and a NOAEL of 300 mg/kg/day for mice (Charles et al. 1996b).

Metabolic Effects. Elevated potassium levels were reported prior to death in the case of an individual who may have ingested 25–35 g of 2,4-D from a commercial herbicide product (Keller et al. 1994). Metabolic acidosis was reported in three out of the four nonlethal cases of intoxication with preparations containing 2,4-D reported by Durakovic et al. (1992). No further human information was located.

Limited relevant data are available from studies in animals. Significantly reduced serum calcium and potassium were reported in dogs following administration of a single dose of 200 mg 2,4-D/kg in a gelatin capsule (Dickow et al. 2000). The investigators noted that these effects may have been secondary to vomiting and diarrhea also experienced by the dogs.

No significant alterations in electrolytes or glucose levels were reported in rats dosed with ≤300 mg 2,4-D/kg/day for 13 weeks (Charles et al. 1996a) or ≤150 mg/kg/day for 2 years (Charles et al. 1996b). Also, no significant metabolic alterations were reported in dogs exposed up to ≤7.5 mg 2,4-D/kg/day for 13 weeks, but exposure to ≥5 mg 2,4-D/kg/day for 52 weeks significantly reduced blood glucose (27–31%) in dogs (Charles et al. 1996c).

Based on limited data, it does not appear that metabolic alterations need to be a concern for humans exposed to environmentally levels of 2,4-D.

3.2.2.3 Immunological and Lymphoreticular Effects

No information was located regarding immunological and lymphoreticular effects in humans following oral exposure to 2,4-D.

For the most part, studies in animals only provide information on gross and microscopic morphology of lymphoreticular organs and tissues; limited information is available regarding immunocompetence. No morphological alterations were observed in the spleen and lymph nodes from dogs treated once with up to 125 mg 2,4-D/kg (Steiss et al. 1987).

Intermediate-duration studies did not report morphological alterations in lymphoreticular tissues from rats exposed to ≤300 mg 2,4-D/kg/day (Charles et al. 1996a; EPA 1984, 1985; Gorzinski et al. 1987; Marty et al. 2013). An F1-extended 1-generation study did not find altered immunocompetence (assessed by the SRBC antibody plaque forming cell assay) in the F1 generation that had been exposed directly to ≤75.3 mg 2,4-D/kg/day and indirectly during gestation and lactation (Marty et al. 2013). Results from a natural killer cells assay were also negative. No morphological alterations were reported in mice exposed to ≤90 mg 2,4-D/kg/day (EPA 1984, 1987a) and in dogs exposed to ≤7.5 mg 2,4-D for up to 1 year (Charles et al. 1996c).

Chronic-duration exposure of rats to ≤150 mg 2,4-D/kg/day (Charles et al. 1996b; Hansen et al. 1971), mice to ≤300 mg 2,4-D/kg/day (Charles et al. 1996b; EPA 1987a), or dogs to ≤10 mg 2,4-D/kg/day (Hansen et al. 1971) did not result in gross or microscopic alterations in lymphoreticular organs or tissues.

The available animal data, although rather limited, suggest that immunological alterations should not be a concern for humans exposed to environmental levels of 2,4-D.

NOAEL and LOAEL values for immune system effects in each species and duration category are recorded in Table 3-2 and plotted in Figure 3-2.

3.2.2.4 Neurological Effects

Neurological effects have been reported in most cases of intoxication with commercial products containing 2,4-D. For example, coma and absence of reflexes were reported on admission in three out of the four nonlethal cases of intoxication described by Durakovic et al. (1992). The lethal case reported by Dudley and Thapar (1972) was described as comatose upon admission to the emergency room. Autopsy of the latter revealed multiple petechiae throughout the white matter of the brain. However, microscopic examination of the brain showed changes (i.e., senile plaques, lipofuscin accumulation) that appeared consistent with senile dementia (the subject was 76 years old) and not caused by the acute intoxication. Internal examination of another lethal case showed slight edema of the brain and pia-arachnoid (Nielsen et al. 1965). Histological examination showed marked congestion at all brain levels examined as well as severe degenerative changes in ganglion cells. Information regarding signs and symptoms before death was not available because the subject was found dead in an uninhabited area. Because the time elapsed between death and the postmortem examination was unknown, it is impossible to determine with certainty whether the histological alterations seen in the brain were caused by the product ingested or represented normal postmortem changes. Neurological examination of a man 24 hours after ingesting approximately 110 mg 2,4-D/kg from a commercial herbicide product showed hyperactive biceps and triceps, but no other abnormal reflexes; the subject, however, did complain of hyperesthesia of the upper part of his torso (Berwick 1970).

Numerous studies in animals provide information on gross and microscopic morphology in the nervous system following exposure to 2,4-D; a few studies also examined neurobehavioral parameters. In general, the results show lack of adverse morphological effects at the dose levels tested, but some studies reported neurobehavioral and neurochemical alterations.

An acute-duration study reported that a single gavage dose of 300 mg 2,4-D/kg induced vascular damage in the central nervous system in rats; no such effect was observed at 150 mg 2,4-D/kg (Elo et al. 1988). The effect was attributed to 2,4-D-induced damage to the blood brain barrier, caused in turn by saturation of the organic acid transport out of the brain. A single lower dose of 250 mg 2,4-D/kg administered to rats did not induce morphological alterations in the brain, spinal cord, or trigeminal nerve (Mattsson et al. 1997). Also, no morphological alterations were reported in the brain or spinal cord from dogs given a single dose of up to 125 mg 2,4-D/kg in a capsule (Steiss et al. 1987).

Intermediate-duration studies in rats did not report morphological alterations in tissues of the nervous system even with the highest doses tested, 300 mg 2,4-D/kg/day (Charles et al. 1996a). Other studies that examined this end point in rats include EPA (1984, 1987a), Gorzinski et al. (1987), Marty et al. (2013), and Mattsson et al. (1997). No significant morphological alterations in the nervous system were reported in mice exposed to ≤90 mg 2,4-D/kg/day (EPA 1984, 1987a) or dogs exposed to ≤7.5 mg 2,4-D/kg/day (Charles et al. 1996c).

No morphological alterations in the nervous system were reported in chronic-duration studies in rats administered ≤150 mg 2,4-D/kg/day (Charles et al. 1996b), mice exposed to ≤300 mg 2,4-D/kg/day (Charles et al. 1996b; EPA 1987a), or dogs exposed to ≤10 mg 2,4-D/kg/day (Hansen et al. 1971).

Studies have also examined neurobehavioral parameters in animals following oral exposure to 2,4-D. In fact, the lowest LOAEL for neurological effects in animals was 15 mg 2,4-D/kg (lowest dose tested) for alterations in maternal behavior in rats dosed via the food on postpartum days 1–7 (Stürtz et al. 2008). Specifically, the effects consisted of increased latency of retrieval of pups, increased latency of crouching, decreased percent dams licking the pups, decreased percent dams licking the anogenital region of the pups, increased percent of dams leaving the nest, and increased time spent out of the nest. These behaviors were associated with a decrease in serotonin and an increase in dopamine in the arcuate nucleus of the brain. The relevance of these behavioral effects to humans is unknown. Much higher doses (250 mg 2,4-D/kg, but not 75 mg/kg) induced altered gait and increased motor activity in rats 1 day after dosing (Mattsson et al. 1997), and a single dose of 125 mg 2,4-D/kg (highest dose tested) did not affect motor nerve conduction velocity in dogs (Steiss et al. 1987).

In intermediate-duration studies, results from tests for motor activity, acoustic startle response, and a functional observational battery (FOB) administered to 54–56-day-old rats exposed to 59.2–81.7 mg

2,4-D/kg/day in the diet from PND 21 were not significantly different from controls (Marty et al. 2013). It should be mentioned that these rats also had been exposed to 2,4-D *in utero* and through maternal milk. However, higher dietary doses (150 mg 2,4-D/kg/day) administered to adult rats for at least 3 months significantly increased forelimb grip strength; no significant effect was reported at 75 mg/kg/day (Mattsson et al. 1997). In this study, no significant alterations were reported in tests of motor activity or on an FOB. Increased grip strength had also been reported in an earlier study in rats dosed by gavage with ≥20 mg 2,4-D/kg 2 days/week for 5 weeks (Squibb et al. 1983). No neurobehavioral tests were conducted in chronic-duration studies.

Standard tests for neurotoxicity do not suggest that the nervous system is very sensitive to exposure to 2,4-D. The available information also indicates that neurobehavioral effects can be detected before morphological alterations can be observed.

NOAEL and LOAEL values for neurological effects in each species and duration category are recorded in Table 3-2 and plotted in Figure 3-2.

3.2.2.5 Reproductive Effects

Virtually no information was located regarding reproductive effects in humans following oral exposure to 2,4-D. No significant gross or histological alterations were reported in the prostate and testes from a man who died after ingesting at least 80 mg 2,4-D/kg from a commercial herbicide consisting of the dimethylamine salt of 2,4-D (Nielsen et al. 1965).

Numerous studies in animals provide information regarding gross and microscopic appearance of reproductive organs following oral exposure to 2,4-D, but relative few studies provide information regarding other reproductive end points. Overall, the reproductive system does not appear to be a particularly sensitive target for 2,4-D toxicity.

Only one acute-duration study was located (Dinamarca et al. 2007). In that study, administration of ≤100 mg 2,4-D/kg given to pregnant mice on GDs 0–9 did not significantly affect the numbers of corpora lutea, implantation sites, resorptions, or live embryos.

Intermediate-duration studies in which rats were exposed to 2,4-D via the diet did not report gross or microscopic alterations in the reproductive organs from male or female animals (Charles et al. 1996a;

EPA 1984, 1985; Gorzinski et al. 1987; Marty et al. 2013). The highest dose tested was 300 mg 2,4-D/kg/day in a 13-week study (Charles et al. 1996a). A study in which rats were administered 2,4-D daily by gavage for 30 days reported histological alterations in Sertoli and Leydig cells even with the lowest dose tested (50 mg/kg/day) (Joshi et al. 2012). The only plausible explanation for the discrepancy in results from Joshi et al. (2012) and those reported in other studies is the different mode of administration of 2,4-D (gavage versus diet).

Fertility was not affected in male or female rats exposed to up to 111 mg 2,4-D/kg/day in intermediate-duration studies (EPA 1986; Hansen et al. 1971; Marty et al. 2013; Saghir et al. 2013), and neither were mating index, time to mating, gestation length, pre- and postimplantation losses, and number of corpora lutea in rats exposed to ≤50 mg 2,4-D/kg/day (Marty et al. 2013). Sperm parameters were also not affected in the latter study, but sperm count and motility were significantly reduced in rats exposed to ≥50 mg 2,4-D/kg/day in the 30-day gavage study mentioned above (Joshi et al. 2012). In addition, serum levels of testosterone, follicle-stimulating hormone, and luteinizing hormone were significantly reduced in male rats (only males tested) from the Joshi et al. (2012) study.

Additional intermediate-duration studies did not report morphological alterations in the reproductive organs from mice exposed via the diet to up to 45 mg 2,4-D/kg/day for 52 weeks (EPA 1987a) or 90 mg 2,4-D/kg/day for 13 weeks (EPA 1984), or in dogs exposed to up to 7.5 mg 2,4-D/kg/day for 1 year (Charles et al. 1996c).

Two-year dietary studies also did not report morphological alterations in the reproductive organs from rats exposed to up to 150 mg 2,4-D/kg/day (Charles et al. 1996b; Hansen et al. 1971), mice exposed up to 300 mg 2,4-d/kg/day (Charles et al. 1996b; EPA 1987a), or dogs exposed up to 10 mg 2,4-D/kg/day (Hansen et al. 1971).

2,4-D did not induce adverse reproductive effects in animals when administered via the diet, at the dietary levels tested. However, a gavage study reported histopathology of the testes and alterations in sperm parameters and serum levels of reproductive hormones (Joshi et al. 2012). The available data suggest that exposure to environmental levels of 2,4-D by a relevant route is unlikely to cause adverse reproductive effects in humans.

NOAEL and LOAEL values for reproductive effects in each species and duration category are recorded in Table 3-2 and plotted in Figure 3-2.

3.2.2.6 Developmental Effects

No information was located regarding developmental effects in humans following oral exposure to 2,4-D.

Developmental effects have been observed in rodents following perinatal exposure to 2,4-D. For the most part, results from acute-duration studies suggest that effects might be observed at doses that caused maternal effects, mainly reduced maternal weight. For example, exposure of rats to 75 mg 2,4-D/kg/day on GDs 6–15 did not result in significant maternal toxicity or developmental effects in fetuses examined on GD 20 (Charles et al. 2001). However, similar exposure of rats to 100–115 mg 2,4-D/kg/day significantly reduced maternal weight gain during treatment and significantly increased the incidence of morphological and skeletal defects in fetuses examined on GD 20 (Chernoff et al. 1990; Mazhar et al. 2014). In yet similar studies in rats, doses of 70 mg 2,4-D/kg/day during gestation caused maternal weight loss during treatment and induced renal malformations and offspring lethality during the first 2 weeks of life (Fofana et al. 2000, 2002). One study in rats reported significantly reduced fetal weight and increased incidence of soft-tissue and skeletal anomalies on GD 20 following maternal exposure to ≥50 mg 2,4-D/kg/day on GDs 6–15; the NOAEL was 25 mg 2,4-D/kg/day (Schwetz et al. 1971). However, neither growth nor viability were affected in offspring from dams that were allowed to give birth and had been exposed to up to 87.5 mg 2,4-D/kg/day (Schwetz et al. 1971).

Exposure of mice to 87.5 mg 2,4-D/kg/day (only dose level tested) on GDs 8–12 resulted in significantly reduced offspring weight on PND 1, but not PND 3 (Kavlock et al. 1987). While it was noted that there was no significant increases in maternal mortality or resorptions, no information was provided regarding changes in maternal weight during treatment.

No significant developmental effects were reported in hamsters following maternal exposure to up to 100 mg 2,4-D/kg/day on GDs 6–10 (Collins and Williams 1971) or rabbits following maternal exposure to up to 90 mg 2,4-D/kg/day on GDs 6–18 (Charles et al. 2001).

Several intermediate-duration studies provide information on developmental end points; all of the available studies were conducted in rats. The lowest LOAEL for developmental effects was 2.5 mg 2,4-D/kg/day (the lowest dose tested) and this caused a significant reduction in body weight (5–7% on lactation days 10–16) for pups from dams exposed to 2,4-D in the diet on postpartum days 1–16 (Stürtz et al. 2010). This effect was attributed to inhibition of suckling-induced hormone release and milk transfer

to the litter by an action of 2,4-D at the central level. The study also showed that maternal exposure to 2,4-D altered the contents of lipids (30% decreased at 25 mg 2,4-D/kg/day) and of some proteins in the milk. With the changes in milk content, it is possible that nutritional deficiency occurred that resulted in hindered growth of the pups. Other studies have also reported effects on pup body weight, but at higher 2,4-D doses. For example, in a 2-generation reproductive study, pup body weight was reduced significantly on PND 28 at maternal doses ≥35 mg 2,4-D/kg/day during lactation, but not at 10 mg 2,4-D/kg/day (EPA 1986). In another study, reduced pup body weight (about 10%) was reported following perinatal exposure to approximately 9 mg 2,4-D/kg/day on PND 22 (Marty et al. 2013). The study by Stürtz et al. (2010) was used to derive an intermediate-duration oral MRL for 2,4-D. Marty et al. (2013) reported significant decreases in the weight of the adrenals, kidneys, liver, spleen, and testes from pups at the maternal exposure level of approximately 60 mg 2,4-D/kg/day during lactation and sacrificed on PND 22; however, no histological alterations were observed in these organs. Monitoring of developmental landmarks in additional pups born to dams exposed to up to 50 mg 2,4-D/kg/day showed no significant effects on nipple retention in males, age at vaginal opening, or mean estrous cycle length (Marty et al. 2013). There was, however, a slight delay (1.6 days) in the age at preputial separation in male pups, which was attributed to body weight decrement and slightly delayed growth.

Other studies that reported reduced offspring weight at higher maternal 2,4-D doses include Bortolozzi et al. (1999), Hansen et al. (1971), Mazhar et al. (2014), and Troudi et al. (2012a, 2012b). Mazhar et al. (2014) also reported that maternal exposure to 100 mg 2,4-D/kg/day (only dose level tested) on GDs 1–19 significantly increased the incidence of morphological and skeletal defects in fetuses examined on GD 20. Further, exposure to 2,4-D significantly reduced maternal weight gain (40–54%) during treatment and caused decreased activity, rapid breathing, loss of appetite, weakness, nasal hemorrhage, and slight diarrhea.

Other effects that have been reported in intermediate-duration oral studies in rats include neurobehavioral alterations in male and female pups and delayed vaginal opening in females following maternal exposure to 70 mg 2,4-D/kg/day (only dose level tested) (Bortolozzi et al. 1999) and histological alterations in pups' liver and bone following maternal exposure to 126 mg 2,4-D/kg/day (only dose level tested) (Troudi et al. 2012a, 2012b). In the latter two studies, developmental effects were associated with increased markers of oxidative stress and reduced antioxidant enzyme levels in dams and pups.

Overall, studies in animals suggest that 2,4-D does not induce teratogenicity, but it can induce reductions in offspring weight that are not always associated with maternal effects. It has caused alterations in

neurobehavioral effects in one study (Bortolozzi et al. 1999) and inhibited milk ejection at low maternal exposure levels in another study (Stürtz et al. 2010).

NOAEL and LOAEL values for developmental effects in each species and duration category are recorded in Table 3-2 and plotted in Figure 3-2.

3.2.2.7 Cancer

No information was located regarding cancer in humans following oral exposure to 2,4-D.

The potential carcinogenicity of 2,4-D has been examined in bioassays in rats, mice, and dogs, and in these three species, 2,4-D yielded negative results. In these studies, rats were exposed up to 150 mg 2,4-D/kg/day in the diet for 2 years (Charles et al. 1996b; Hansen et al. 1971), mice were similarly exposed to up to 300 mg 2,4-D/kg/day (Charles et al. 1996b; EPA 1987a), and dogs were exposed up to 10 mg 2,4-D/kg/day for 2 years (Hansen et al. 1971).

2,4-D was not a promoter of liver tumors in rats initiated with diethylnitrosamine for 5 weeks followed by administration of a diet containing 0.05% 2,4-D (approximately 25 mg 2,4-D/kg/day) for 23 weeks (Abdellatif et al. 1990).

Based on the information available, the EPA has assigned 2,4-D to carcinogenicity Group D, "not classifiable as to human carcinogenicity" (EPA 2005a). The Department of Health and Human Services has not classified 2,4-D as to its carcinogenicity (NTP 2014). The International Agency for Research on Cancer (IARC) recently classified 2,4-D as possibly carcinogenic to humans (Group 2B) based on inadequate evidence in humans and limited evidence in experimental animals (IARC 2016; Loomis et al. 2015). IARC has not yet released a full report in support for its recent classification.

3.2.3 Dermal Exposure

As mentioned in the introduction to Section 3.2.1, most of the information available regarding exposure to 2,4-D and health end points in humans comes from studies of individual occupationally exposed either through farming activities or manufacture, formulation, or packaging of herbicide products containing 2,4-D. In these activities, exposure is likely to be predominantly by dermal contact with products containing 2,4-D, with inhalation exposure playing a lesser role. Therefore, studies of humans involved in these activities are summarized in this section. However, the reader should keep in mind that the health

outcomes described are the result of exposure through multiple routes, usually a combination of inhalation, oral, and dermal.

3.2.3.1 Death

Cause-specific mortality was examined among employees engaged in the manufacture, formulation, or packaging of 2,4-D and related salts. Three studies were published, the original report (Bond et al. 1988), a 4-year follow-up (Bloemen et al. 1993), and a subsequent assessment of mortality to the end of 1994 (Burns et al. 2001). Various industrial plants were involved, and potential exposure to other chemicals was likely to have occurred based on the plant, the period, and the job; however, the common factor for the cohort was potential exposure to 2,4-D. Exposure data were provided in the first report and ranged from an estimated time-weighted average (TWA) of 0.18 to 3 mg/m^3 2,4-D for the various job categories. The first report included 878 chemical workers and the most recent report involved 1,515 male employees who contributed 39,799 person-years at risk for an average follow-up of 26.2 years. In none of the three studies were there patterns suggestive of a causal association between exposure to 2,4-D and any particular cause of death, including NHL, which has received the most attention in relation to exposure to phenoxy herbicides. Bloemen et al. (1993) calculated a Standardized Mortality Ratio (SMR) of 196 (95% confidence interval [CI] 24–708) and Burns et al. (2001) calculated an SMR of 1.0 (95% CI 0.21–292) for NHL in the studies.

Many additional studies have examined mortality rates in subjects exposed to herbicides, particularly phenoxy herbicides that included 2,4-D, but did not conduct analyses for individual chemicals. Some examples of such studies include Becher et al. (1996), Bueno de Mesquita et al. (1993), Coggon et al. (1991), Gambini et al. (1997), Green (1991), Riihimäki et al. (1982), Saracci et al. (1991), Thörn et al. (2000), and Zahm (1997). Cohort sizes ranged from a few hundred subjects (Thörn et al. 2000) to >30,000 subjects in a study of employees of a lawn care service company (Zahm 1997). Except for the Zahm (1997) study, none of these studies found significantly elevated mortality risks for NHL. Zahm (1997) reported a significantly elevated SMR of 7.11 (95% CI 1.78–28.42) based on two cases of NHL among male applicators employed in the lawn care service company for >3 years. Although it could not be concluded that the NHL risk was related to exposure to pesticides or to a specific product such as 2,4-D, it was the only tumor with a duration effect; the SMR of 7.11 was similar to higher risk seen in frequent herbicide users in other studies (see Section 3.2.3.7, Cancer).

The only information available from studies in animals is that the dermal LD_{50} in rabbits was determined to be >2,000 mg/kg (Gorzinski et al. 1987).

3.2.3.2 Systemic Effects

No information was located regarding cardiovascular, musculoskeletal, or ocular effects in humans following dermal exposure to 2,4-D. No information was located regarding respiratory, gastrointestinal, cardiovascular, musculoskeletal, endocrine, or metabolic effects in animals following dermal exposure to 2,4-D. The highest NOAEL values and all LOAEL values from each reliable study for other systemic effects in each species and duration category are recorded in Table 3-3.

Respiratory Effects. In the Agricultural Health Study (AHS), use of 2,4-D was not associated with wheezing (odds ratio [OR] 0.97; 95% CI 0.86–1.10 for farmers; OR 0.99; 95% CI 0.73–1.34 for applicators) (Hoppin et al. 2006a, 2006b). The AHS is a prospective cohort study of nearly 90,000 private pesticide applicators (mostly farmers), their spouses, and commercial pesticide applicators in Iowa and North Carolina. The AHS is sponsored by the National Institutes of Health (NIH 2014). In the study, exposure and outcome were assessed using two self-administered questionnaires that provided information regarding 40 specific chemicals (2,4-D among them) used in the year before enrollment, pesticide application methods, current agricultural activities, smoking history, medical history, and demographics. In the AHS, use of 2,4-D was associated with current rhinitis (OR 1.34; 95% CI 1.09–1.64) (Slager et al. 2009). However, further analysis showed that rhinitis was associated only with current use of both 2,4-D and glyphosate, while current use of either herbicide alone was not associated with rhinitis when modeled separately (OR 0.99; 95% CI 0.63–1.54 for 2,4-D alone). In addition, analysis by days/years applied showed no dose-response relationship for 2,4-D. In a group of 583 farm women in the AHS, prevalence of self-reported history of doctor-diagnosed chronic bronchitis was associated with lifetime exposure to 2,4-D in models adjusted for age and state (OR 1.29; 95% CI 1.02–1.63) (Valcin et al. 2007). No association was found following multivariate adjustment that added variables within the herbicide group (OR 1.20: 95% CI 0.89–1.63). A similar study of farm women in the AHS found that use of 2,4-D was associated with self-reported history of atopic asthma (OR 1.53; 95% CI 1.12–2.10), but not with nonatopic asthma (OR 1.07; 95% CI 0.82–1.41) (Hoppin et al. 2008).

Table 3-3 Levels of Significant Exposure to 2,4-Dichlorophenoxyacetic Acid - Dermal

Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL	LOAEL				Reference Chemical Form	Comments
				Less Serious		Serious			
ACUTE EXPOSURE									
Death									
Rabbit (New Zealand)	24 hr (GO)					2000 B mg/kg	(LD50)	Gorzinski et al. 1987 2,4-dichlorophenoxyacetic acid	The LD50 was greater than 2000 mg/kg.
Systemic									
Dog hairless	7 d 1 x/d	Dermal		0.036 mg	(slight epidermal thickening and hyperplasia)			Kimura et al. 1998 2,4-dichlorophenoxyacetic acid	
Rabbit (New Zealand)	4 hr	Dermal	500 B mg					EPA 1992 2,4-dichlorophenoxyacetic acid	NOAEL is for skin irritation.
Immuno/ Lymphoret									
Mouse (BALB/c)	9 d			5 F Percent (%)	(respiratory allergen)			Fukuyama et al. 2009	

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Table 3-3 Levels of Significant Exposure to 2,4-Dichlorophenoxyacetic Acid - Dermal (continued)

Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL	LOAEL			Reference Chemical Form	Comments
				Less Serious		Serious		
INTERMEDIATE EXPOSURE								
Systemic								
Rabbit (New Zealand)	21 d 7 d/wk 6 h/d	Hemato	1000 B mg/kg/day				EPA 1991a 2,4-dichlorophenoxyacetic acid	
		Hepatic	1000 B mg/kg/day					
		Renal	100 F mg/kg/day	1000 F mg/kg/day	(increased absolute and relative kidney weight)			
		Dermal		10 F mg/kg/day	(very slight erythema)			
		Ocular	1000 B mg/kg/day					
		Bd Wt	1000 B mg/kg/day					

B = both sexes; Bd Wt = body weight; d = day(s); F = Female; (GO) = gavage in oil; Hemato = hematological; hr = hour(s); LD50 = lethal dose, 50% kill; LOAEL = lowest-observed-adverse-effect level; NOAEL = no-observed-adverse-effect level; x = time(s); wk = week(s)

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Gastrointestinal Effects. Nausea and vomiting were reported in two cases of intoxication due to dermal contact with an herbicide containing 2,4-D (Goldstein et al. 1959). No further relevant information was located.

Hematological Effects. Hemoglobin concentration and erythrocyte and leukocyte counts were within normal limits in three cases of intoxication due to dermal contact with an herbicide containing 2,4-D (Goldstein et al. 1959).

Intermittent application of up to 1,000 mg 2,4-D/kg/day onto the back of rabbits for 21 days did not induce treatment-related alterations in hematological parameters (EPA 1991a). No further information was located.

Hepatic Effects. Schreinemachers (2010) conducted a study of a subset of 727 healthy participants from the cross-sectional National Health and Nutrition Examination Survey (NHANES), 1988–1994, 20–59 years of age, to investigate risk factors that are linked to the pathogenesis of acute myocardial infarction and type-2 diabetes soon after exposure to 2,4-D. Only 14% of the subjects had urinary 2,4-D levels above the limit of detection (1 mg/dL). Subjects with urinary 2,4-D level above and below the detection level were compared. The results showed that subjects with detectable urinary 2,4-D had significantly lower serum high-density lipoprotein (HDL) than subjects with undetectable 2,4-D in the urine, although still within the normal range. No significant differences were observed between the groups for serum triglycerides and non-HDL cholesterol levels. The investigators also noted that in susceptible populations characterized by high serum glucose and low T4, 2,4-D was associated with increased levels of serum triglycerides. Because no formal statistical sampling procedure was used to recruit the subset of NHANES volunteers, the cohort was not representative of the U.S. population. In addition, it was not clearly indicated in the study when the urine and serum samples were collected in relation to the exposure to 2,4-D or whether there could have been exposure to other chemicals.

Results from a sulfobromophthalein test for liver function performed in one of the cases of dermal intoxication reported by Goldstein et al. (1959) were normal. It is unclear whether liver tests were performed on the two other cases described in the report.

The only relevant information in animals is that application of up to 1,000 mg 2,4-D/kg/day onto the skin of rabbits for 21 days did not induce treatment-related alterations in clinical chemistry tests or histological alterations in the liver (EPA 1991a).

Renal Effects. In the cross-sectional study mentioned above (Schreinemachers 2010), subjects with measurable urinary levels of 2,4-D had significantly higher levels of urinary creatinine than subjects with undetectable levels, but still within the normal range. In the absence of additional renal function tests, the biological significance of this finding is unknown.

Urinalysis was normal in one of the cases of dermal exposure to an herbicide containing 2,4-D described by Goldstein et al. (1959). In another case, urinalysis showed persistent albuminuria and occasional casts (Goldstein et al. 1959).

Application of 10–1,000 mg 2,4-D/kg/day onto the skin of male and female rabbits for 21 days significantly increased absolute and relative kidney weight in high-doses females (EPA 1991a). However, while kidney function tests and histology were performed, there were no treatment-related alterations in clinical chemistry for kidney function nor histological changes in the kidneys.

Endocrine Effects. Mean serum levels of T4, thyroid-stimulating hormone (TSH), insulin, and C-peptide (a marker of endogenous production of insulin) in a group of 102 subjects with detectable levels of 2,4-D in the urine were not different from those in 625 subjects with urinary 2,4-D below the limit of detection (1 mg/dL) (Schreinemachers 2010). However, in subjects with low HDL, 2,4-D was associated with increased levels of C-peptide ($p \leq 0.05$), insulin ($p \leq 0.01$), and TSH ($p \leq 0.05$), especially in populations with high serum glucose and low T4 levels.

Additional information regarding endocrine effects is available from the AHS. Goldner et al. (2010) examined 16, 529 female spouses of pesticide applicators who had thyroid data, pesticide use data, and all covariates data. Among this group, 2.2% classified as hyperthyroid, 6.7% as hypothyroid, 3.4% as having other thyroid disease, and 87.6% as having no thyroid disease. Regression analyses showed elevated ORs for hypothyroid disease if the spouse ever worked or lived on a farm (OR 1.3; 95% CI 0.87–2.0). Analyses of individual pesticides yielded an OR of 0.93 (95% CI 0.68–1.3) for ever-use of 2,4-D and hyperthyroidism, an OR of 0.96 (95% CI 0.8–1.1) for hypothyroidism, and an OR of 1.2 (95% CI 0.95–1.5) for other thyroid disease. In a subsequent study of male participants in the AHS, Goldner et al. (2013) reported a positive association between ever-use of 2,4-D and hypothyroid disease (OR 1.35;

95% CI 1.04–1.76). Exposure-response analyses using the intensity-weighted measure showed a monotonic exposure-response for 2,4-D. The seemingly conflicting results between the study of women and the one of men may reflect, at least in part, the fact that male pesticide applicators use a larger number of pesticides and often apply larger amounts of individual pesticides than their female spouses, as noted by Goldner et al. (2013).

Dermal Effects. The only relevant information available is that of a case in which a farmer who accidentally wetted his legs with an herbicide containing 2,4-D developed desquamation of the skin of the palms and soles (Goldstein et al. 1959).

Limited information is available regarding dermal effects of 2,4-D in animals. Hairless dogs that received daily application of a 0.036 mL of a 0.1% solution of 2,4-D for 7 days showed no inflammation or pigmentation at the application site 1 day after termination of dosing (Kimura et al. 1998). No gross changes were seen 14 days after cessation of dosing. One day after cessation of treatment, light microscopy showed slight epidermal thickening and hyperplasia; no significant changes were seen 14 days after termination of treatment. The skin of rabbits that received an application of 0.5 g of 2,4-D onto a shaved area of the skin for 4 hours did not show signs of irritation (EPA 1992). Repeated application of ≥10 mg 2,4-D/kg/day to the skin of rabbits for 21 days resulted in slight erythema and epidermal scaling at various times during the study, but no edema was observed (EPA 1991a).

Ocular Effects. The only information regarding ocular effects in humans following exposure to 2,4-D is that from a study of 31,173 wives whose husbands were licensed pesticide applicators participating in the AHS (Kirrane et al. 2005). Using logistic and hierarchical logistic regression analyses after adjusting for potential effect modifying and potential confounders, an OR of 1.1 (95% CI 0.7–1.8) was reported for use of 2,4-D and retinal degeneration or other eye disorders.

The only relevant information in animals is that application of up to 1,000 mg 2,4-D/kg/day onto the skin of rabbits for 21 days did not induce histological alterations in the eyes (EPA 1991a).

Body Weight Effects. Significant weight loss (~9 kg) was reported in two cases of dermal exposure to herbicide products containing 2,4-D (Goldstein et al. 1959). One of the cases had experienced nausea and vomiting for about 10 days after exposure, which could explain, at least in part, the weight loss. The other patient had been affected by anorexia while hospitalized due to adverse neurological symptoms.

A study that included 8,365 male pesticide applicator participants in the AHS examined the relationship between total cumulative exposure from age 20 years to the time of 5-year follow-up to classes of pesticides and individual components and body mass index (BMI) (LaVerda et al. 2015). Results from unadjusted and adjusted regression models that maintained all covariates in models estimating the association between exposure and amount of BMI associated with 100 cumulative exposure days between age 20 and age at follow-up showed a positive association for 2,4-D for Iowa applicators (p=0.0258 and 0.0183, respectively). However, after medical exclusions (cancer excluding non-melanoma skin cancer, diabetes, heart disease, lupus, and/or amyotrophic lateral sclerosis), no significant associations remained (p=0.2408).

Body weight was not significantly affected in rabbits that received intermittent applications of up to 1,000 mg 2,4-D/kg/day for 21 days (EPA 1991a).

Metabolic Effects. In a cross-sectional study of a subset of NHANES 1988–1994 subjects, serum levels of glucose and glycosylated hemoglobin (marker for mean plasma concentration of glucose over a prolonged period of time) in a group of 102 subjects with detectable levels of 2,4-D in the urine were not different from mean levels recorded in 625 subjects with urinary 2,4-D below the limit of detection (1 mg/dL) (Schreinemachers 2010).

3.2.3.3 Immunological and Lymphoreticular Effects

No studies were located that examined a potential association between exposure specifically to 2,4-D and immunological parameters in humans. A small study of 10 Italian farmers reported that exposure (assumed to have been acute) to unidentified commercial mixtures containing 2,4-D and 4-chloro-2-methylphenoxy acid (MCPA) resulted in transient alterations in lymphocyte subsets, natural killer cells, and lymphoproliferative response to mitogen stimulations (Faustini et al. 1996). Another study of 47 workers in a plant producing herbicides (2,4-D among them), fungicides, and seed dressings reported alterations in lymphocyte subsets and immunoglobulin A levels compared to unexposed control individuals (Kluciński et al. 2001). However, neither of these studies provided specific information regarding 2,4-D. A nested case-control study of female spouses of participants in the AHS reported an OR of 0.5 (95% CI 0.3–0.9) for exposure to 2,4-D and rheumatoid arthritis (De Roos et al. 2005). There was no explanation for the apparent inverse association.

2,4-D was a respiratory allergen in mice as assessed by a significant increase in total IgE levels and IgE-expressing B-cell populations following repeated dermal applications of 25 µL of a 5% solution of 2,4-D in acetone/saline (doses of approximately 62.5 mg 2,4-D/kg) and then challenged intratracheally with 50 µL of a 0.5% solution of the chemical (Fukuyama et al. 2009). No additional studies were located regarding immunological effects of 2,4-D in animals.

3.2.3.4 Neurological Effects

Information regarding neurological effects in humans following exposure to 2,4-D is limited to a few epidemiological studies and case reports. The epidemiological studies examined the association between pesticide exposure and Parkinson's disease; the results do not suggest a causal association between 2,4-D and the disease. In the AHS, the OR for ever-use of 2,4-D and prevalent cases of Parkinson's disease was 0.9 (95% CI 0.5–1.8), and the OR for incident cases of Parkinson's disease was 1.0 (95% CI 0.5–2.1) (Kamel et al. 2006). Prevalent cases were self-reported cases at enrollment in the AHS, whereas incident cases were self-reported cases at follow-up. A much smaller case-control study of Parkinson's disease in East Texas (100 cases, 84 controls) reported an OR of 1.2 (95% CI 0.6–2.8) for "ever personally used/mixed or applied" 2,4-D and Parkinson's disease (Dhillon et al. 2008). A case-control study of 319 cases of Parkinson's disease and 296 relative and other controls reported an OR of 2.07 (95% CI 0.696.23) for ever-use of 2,4-D and Parkinson's disease (Hancock et al. 2008). A significant association (OR 2.59; 95% CI 1.03–6.48) between use of 2,4-D and risk of parkinsonim was reported in a multicenter case-control study of 519 cases and 511 controls based on 16 cases among exposed subjects and 7 among controls (Tanner et al. 2009).

It should also be mentioned that studies of female spouses of pesticide applicators in the AHS reported that depression (physician-diagnosed or self-reported) was not associated with 2,4-D (Beseler et al. 2006 [OR 1.05, 95% CI 0.99–1.11]; Beard et al. 2013 [RR 0.71; 85% CI 0.58–0.89]). The inverse association reported by Beard et al. (2013) was attributed by the authors to reverse causality or just chance.

Limited data from case reports provide additional information. Goldstein et al. (1959) described three cases of dermal exposure to an herbicide product containing an ester of 2,4-D. In the three cases, there was contact of the product with unprotected skin; symptoms and signs involved the peripheral nervous system and started hours after skin contact with the product containing 2,4-D. In one case, there was a second exposure about 2 months after the first exposure. In general, symptoms consisted of pain, paresthesias (abnormal sensations), and paralysis that were severe enough to require hospitalization of the

three patients. Recovery was slow and some symptoms persisted for years after exposure had occurred. Berkley and Magee (1963) also reported a case of primary sensory neuropathy in a farmer who had dermal contact with a 40% solution of the dimethylamine salt of 2,4-D and water.

No studies were located regarding neurological effects in animals following dermal exposure to 2,4-D acid or simple salts.

3.2.3.5 Reproductive Effects

Limited information is available regarding reproductive effects in humans following exposure to 2,4-D. An early study of 32 male farm sprayers who were exposed to 2,4-D for 1–2 months and 25 controls reported significant differences ($p<0.01$) in various sperm parameters between the exposed and control group, which tended to disappear following a short recovery period; regression analyses were not conducted in this study (Lerda and Rizzi 1991). Although not totally clear, it appears that sperm analyses were conducted 6 months (March) after the exposure period (August–September) and again 3 months later (July) to examine possible recovery. No information was provided regarding possible exposures to other chemicals. A more recent nested case-control study of 50 men with low semen quality and 36 men with sperm parameters within normal limits from Missouri and Minnesota reported an OR of 0.8 (95% CI 0.2–3.0) for levels of 2,4-D in urine (≥0.1 μg/g creatinine) and semen quality (Swan et al. 2003).

A nested case-control study of 2,110 women participants in the Ontario Farm Family Health Study that contributed 3,936 pregnancies including 395 spontaneous abortions found no association between spontaneous abortion and use of 2,4-D during the preconception period (OR 1.2; 95% CI 0.8–1.6) or the post-conception period (OR 1.0; 95% CI 0.7–1.6) (Arbuckle et al. 2001). However, when models were constructed with exposure window as the outcome, preconception exposure to 2,4-D was associated with increased risk of early abortion (<12 weeks) (OR 2.9; 95% CI 1.1–8.0), but not with risk of late spontaneous abortion (OR 0.5; 95% CI 0.2–1.1). A prior study of this population, that did not control for history of prior spontaneous abortion, did not find associations between exposure to 2,4-D and spontaneous abortions (Arbuckle et al. 1999); the OR for preconception exposure adjusted for maternal age, education, and alcohol intake was 0.9 (95% CI 0.5–1.8) and the OR for postconception exposure was 1.1 (95% CI 0.5–2.4). The available data are insufficient due to multiple factors, one being the likelihood of being exposed to a mixture of pesticides, to determine whether exposure to 2,4-D can adversely affect reproductive function in humans.

No studies were located regarding reproductive effects in animals following dermal exposure to 2,4-D.

3.2.3.6 Developmental Effects

A case-control study of 3,412 pregnancies and 118 malformations nested in the Ontario Farm Family Health Study did not find associations between exposure to 2,4-D and birth defects (Weselak et al. 2008). The investigators performed separate analyses for reported use of 2,4-D during the preconception period (OR 1.07; 95% CI 0.55–2.08) and during the post-conception period (OR 0.97; 95% CI 0.42–2.25), and for couples who lived on farms where the father had reported direct chemical activity during a relevant period of time and there was reported use of 2,4-D (OR 0.60; 95% CI 0.25–1.46). A similar study examined the potential associations between women's residential proximity to agricultural pesticide applications in the San Joaquin Valley of California during early pregnancy and risk of neural tube defects and orofacial clefts (Yang et al. 2014). Evaluation of the association between exposure to a mixture of 2,4-D and dichlorprop and risk of anencephaly yielded an OR of 2.0 (95% CI 0.8–51), whereas that between exposure to the mixture and incidence of cleft lip with or without cleft palate produced an OR of 1.1 (95% CI 0.6–2.1). There were too few cases of spina bifida and cleft palate alone for meaningful analyses. A study of 4,935 births to 34,772 state-licensed, private pesticide applicators in Minnesota found that in regions where chlorophenoxy herbicides and/or fungicides were frequently used, infants conceived in spring, when application of the chemicals routinely occurred, showed an increase in birth defects compared to infants conceived in other seasons (OR 1.36; 95% CI 1.10–1.69) (Garry et al. 1996); chemical-specific analyses were not conducted in this study. The same group of investigators conducted a follow-up study of 695 farm families and 1,532 children from the same area in Minnesota during 1997–1998. This study confirmed the earlier finding that conceptions in the spring led to significantly more children with birth defects compared with children conceived in any other season (p=0.02; ORs were not estimated), but chemical-specific analyses were not conducted (Garry et al. 2002).

Evaluation of morbidity among children born to participants in the Ontario Farm Family Health Study reported an increased risk of hay fever or allergies associated with maternal exposure to 2,4-D during pregnancy (Weselak et al. 2007). ORs were estimated as 1.84 (95% CI 1.08–3.04) for male offspring and 1.26 (95% CI 0.70–2.28) for female offspring. No increased risks were reported for asthma or persistent cough or bronchitis. Evaluation of birth weight among 2,246 farm women in the AHS whose most recent singleton birth occurred within 5 years of enrollment (1993–1997) showed that ever-use of 2,4-D during early pregnancy was associated with a reduction of 38 grams in birth weight(95% CI [-103]–27)

(Sathyanarayana et al. 2010). The limited data available, with mostly mixtures or unclear exposure to 2,4-D, do not suggest a role for 2,4-D in birth defects or other developmental effects in humans.

No studies were located regarding developmental effects in animals following dermal exposure to 2,4-D.

3.2.3.7 Cancer

Cancers Affecting the Lymphatic System. Many studies, mostly population-based, case-control design, have examined the relationship between phenoxy herbicides and cancers affecting the lymphatic system, especially NHL. However, only a relatively small number provided information regarding specific products such as 2,4-D.

NHL. Several studies reported increased risk of NHL associated with exposure to 2,4-D. In a population-based, case-control study in Kansas, ever-use of phenoxyacetic acids, mostly 2,4-D, was associated with an OR of 2.2 (95% CI 1.2–4.1) based on 24 cases and 78 controls (Hoar et al. 1986). Use of 2,4-D only was associated with an OR of 2.6 (95% CI 1.4–5.0) based on 21 cases and 60 controls. Stratification by duration of use, frequency of use, and latency did not show consistent dose-responses, but those with the highest frequency of use (≥21 days/year) had the highest OR of 7.6 (95% CI 1.8–32.3), although stratification resulted in small number of cases and controls. A Canadian multicenter population-based, case-control study of 517 cases and 1,506 controls reported an increased OR for phenoxyherbicides and specifically for exposure to 2,4-D (OR 1.32; 95% CI 1.01–1.73) and mecoprop (MCPP), but not for other phenoxyherbicides (McDuffie et al. 2001). Stratification of the subjects by the number of days per year of exposure, however, did not show a dose-response relationship. A nested case-control study embedded in a cohort of 139,000 ever-members of a farm worker labor union in California reported an increased risk of NHL and high use of 2,4-D (OR 3.80; 95% CI 1.85–7.81) (Mills et al. 2005). Prevalence of exposure, however, was low (only 15% for 2,4-D). The investigators noted also that since cases and controls were not interviewed in the study and only work histories were available, no information was collected for parameters that may be involved in the etiology of lymphohematopoietic cancers such as smoking history, diet, or medical history. Hardell et al. (1994) also reported an increased risk of NHL with exposure to 2,4-D (OR 13; 95% CI 1.2–360) in a case-control study of 105 NHL cases and 335 controls based on only three cases and one control. An Italian multicenter case-control study of 1,145 NHL cases and 1,232 controls found that overall use of 2,4-D was not associated with NHL (OR 0.9; 95% CI [0.5–1.8]) (Miligi et al. 2006). However, an increased risk (OR 4.4; 95% CI 1.1–29.1)

was reported among subjects who used 2,4-D but never used protective equipment, based on nine cases and three controls, suggesting that they actually had the highest exposure in this study (Miligi et al. 2006).

Some studies have not found statistically significant associations between NHL and agricultural exposure to 2,4-D (Cantor et al. 1992 [OR 1.2; 95% CI 0.9–1.6]; De Roos et al. 2003 [OR 0.8; 95% CI 0.6–1.1]; Lee et al. 2004b [OR 1.0; 95% CI 0.8–1.3]; Weisenburger 1990 [OR 1.5; 95% 0.9–2.4]; Woods et al. 1987 [OR 0.68; 95% CI 0.3–1.4]; Zahm et al. 1990 [OR 1.5; 95% CI 0.9–2.5]), residential use of 2,4-D (RR 0.89; 95% CI 0.49–1.59) (Hartge et al. 2005), exposure during manufacture (Burns et al. 2011; Standardized Incidence Ratio [SIR] 1.36 [95% CI 0.74–2.29]), or in children from parents in Iowa participating in the AHS (Flower et al. 2004 [OR 1.18; 95% CI 0.29–4.70]). However, in the Burns et al. (2011) study, duration and cumulative exposure to 2,4-D elevated the relative risk 2–3-fold. No associations were reported in a few studies that did not assess 2,4-D alone, but assessed the combination of 2,4-D and other phenoxy acids such as 2,4,5-T (Eriksson et al. 2008 [OR 1.61; 95% CI 0.87–2.97]; Fontana et al. 1998 [OR 1.5; 95% CI 0.4–5.8]; Hardell and Eriksson 1999 [OR 1.3; 95% 0.7–2.3]), or 2,4-DP and 2,4-DB (Kogevinas et al. 1995 [OR 1.11; 95% CI 0.46–2.65]). A meta-analysis that evaluated the weight of evidence of the epidemiological studies of NHL did not find evidence that would support an association between exposure to 2,4-D and NHL (rate ratio [RR] 0.97; 95% CI 0.77–1.22) (Goodman et al. 2015).

Hodgkin's Disease. No association was found between 2,4-D and Hodgkin's disease in case-control studies conducted in the United States (Hoar et al. 1986 [OR 0.8; 95% CI 0.5–1.2]) and Canada (Pahwa et al. 2006 [OR 0.96; 95% 0.67–1.37]), or in a case-control study in Italy that assessed combined exposed of 2,4-D and 2,4,5-T (ORs were not estimated) (Fontana et al. 1998). Among children of parents in Iowa participating in the AHS, Hodgkin's disease cases diagnosed at 0–19 years of age were elevated (OR 2.56; 95% CI 1.06–6.14) based on five cases observed and 1.96 expected (Flower et al. 2004). However, analyses for specific products showed that neither maternal ever-use of 2,4-D (n=3,009, OR 0.72 [95% CI 0.32–1.60]) nor prenatal paternal use of 2,4-D (n=8,769, OR 1.29 [95% CI 0.71–2.35]) was associated with childhood cancer (Flower et al. 2004).

Soft Tissue Sarcoma (STS). In the population-based, case-control study of Hoar et al. (1986), exposure to 2,4-D was not associated with STS; an OR was not provided in the publication. A study of 357 cases and 1,506 controls residents of one of six Canadian provinces found no significant association between exposure to 2,4-D and STS (OR 0.97; 95% CI 0.71–1.32) (Pahwa et al. 2006). Restricting the analysis to 156 farm/dwelling/working cases and 673 controls yielded an OR of 0.96 (95% CI 0.63–1.47). STS was

not elevated among 17,357 children (0–19 years of age) of parents in Iowa participating in the AHS (SIR 1.11; 95% [CI 0.38–3.62]) (Flower et al. 2004). Neither maternal ever-use of 2,4-D (n=3,009, OR 0.72 [95% CI 0.32–1.60]) nor prenatal paternal use of 2,4-D (n=8,769, OR 1.29 [95% CI 0.71–2.35]) was associated with childhood cancer (Flower et al. 2004). A case-control study nested in a large international cancer mortality study of workers exposed to phenoxy herbicides, chlorophenols, and dioxins (Kogevinas et al. 1997), reported an increased risk of STS (OR 5.72; 95% CI 1.14–28.65) for workers exposed to 2,4-D/2,4-DP/2,4-DB based on 9 cases and 24 controls (Kogevinas et al. 1995). Stratification by exposure category (none, low, medium, and high) resulted in dose-related associations; respective ORs were 4.55 (95% CI 0.61–53.4), 6.13 (95% CI 0.33–129.7), and 13.71 (95% CI 0.90–309).

Multiple Myeloma. No association has been found between agricultural exposure to 2,4-D and multiple myeloma in the few studies that examined this possibility (Brown et al. 1993 [OR 1.0; 95% CI 0.6–1.6]; Mills et al. 2005 [no data presented]; Pahwa et al. 2006 [OR 1.21; 95% CI 0.89–1.68]).

Leukemia. Risk of leukemia was reduced (OR 0.55; 95% CI 0.15–2.06) among males in association with 2,4-D in a study of lymphohematopoietic cancers among farmers in California (Mills et al. 2005). In females, the risk was elevated (OR 3.73; 95% CI 0.77–18.0), although the prevalence of exposure to 2,4-D was only 15% in this study. Childhood leukemia was not associated with exposure to 2,4-D in house dust (OR 0.96; 95% CI 0.85–1.08) in a study of 269 cases and 333 healthy controls (Metayer et al. 2013). No association was reported between agricultural exposure to 2,4-D and leukemia (OR 1.2; 95% CI 0.9–1.6) in a case-control study of men in Iowa and Minnesota (Morris et al. 1990). The standardized incidence ratio (SIR) for leukemia was not elevated (SIR 0.91; 95% CI 0.47–1.75) among 17,357 children (0–19 years of age) from parents in Iowa participating in the AHS (Flower et al. 2004). Neither maternal ever-use of 2,4-D (n=3,009, OR 0.72 [95% CI 0.32–1.60]) nor prenatal paternal use of 2,4-D (n=8,769, OR 1.29 [95% CI 0.71–2.35]) was significantly associated with childhood cancer (Flower et al. 2004).

Gastrointestinal Cancer. A few studies provided information regarding 2,4-D and cancer to the gastrointestinal tract; the findings have been mixed. A small study of 72 colon cancer cases diagnosed in Kansas during 1976–1982 and 948 controls selected from the general population found an increased risk for farmers exposed to phenoxy herbicides than to other chemical groups (Hoar et al. 1985). The OR based on six cases that reported use of 2,4-D was 2.0 (95% CI 0.6–6.3), and two of the six cases also reported exposure to 2,4,5-T. The AHS reported an inverse association between ever/never exposed to 2,4-D by pesticide applicators and risk of colorectal cancer (OR 0.7; 95% CI 0.5–0.9) (Lee et al. 2007).

The investigators noted that the lack of a monotonic dose-response pattern with lifetime exposure weakened the argument for a true protective relationship.

A population-based, case-control study of 170 men and women diagnosed with stomach cancer or 137 diagnosed with esophageal cancer and 502 controls in eastern Nebraska did not find an association with ever-use of 2,4-D (OR 0.8; 95% CI 0.4–1.3 for stomach cancer; OR 0.7; 95% CI 0.4–1.2 for esophageal cancer) (Lee et al. 2004a). However, an earlier case-control study of gastric adenocarcinoma among Swedish workers in various occupations that included 567 cases and 1,165 controls reported an elevated risk after exposure to herbicides (OR 1.56; 95% CI 1.13–2.15) (Ekström et al. 1999). Further analysis showed that the majority of the cases had been exposed to a combination of 2,4-D and 2,4,5-T and only two cases and no controls were exposed to 2,4-D only. The investigators noted that despite the positive association with exposure to phenoxyacetic acids, there was no clear relationship with cumulative duration of exposure. Risk of gastric cancer was increased in a nested case-control study of Hispanic farm workers in California exposed to high levels of herbicides, including 2,4-D, and pesticides (Mills and Yang 2007). The study involved 100 cases and 210 controls. Working in areas with high use of 2,4-D was associated with an increased risk of gastric cancer (OR 1.85; 95% CI 1.05–3.25). However, in multivariate-adjusted analysis using unexposed (zero pounds of use) as the referent category, there was no clear relationship between ORs and pounds of use. Moreover, gastric cancer risk was elevated only for pounds of use (1–14 pounds) in the second quartile, but not for the third (15–86 pounds) or the fourth quartile (86–1950 pounds). The investigators noted that not collecting information on dietary habits, family history, smoking, or alcohol consumption may have confounded the results.

Breast Cancer. A nested case-control study of newly diagnosed cases was conducted within a cohort of Hispanic women farm workers in California who were members of the United Farm Workers (UFW) of America (Mills and Yang 2005). The study included 128 cases diagnosed in 1988–2001 and 640 cancer-free controls. Cases included all newly diagnosed invasive breast cancers diagnosed among past or present members of the UFW between 1987 and 2001. The women were exposed to multiple pesticides. ORs for risk of breast cancer associated with pounds of use of all chemicals combined showed increases in multivariate-adjusted analyses. Adjusted ORs for breast cancer in quartiles of pesticide used were 1.00, 1.30 (95% CI 0.73–2.30), 1.23 (95% CI 0.67–2.27), and 1.41 (95% CI 0.66–3.02). Analyses for individual chemicals stratified by year of diagnosis (early, 1988–1994; late, 1995–2001) showed an elevated risk only for high 2,4-D use in late-diagnosed cases (OR 2.14; 95% CI 1.06–4.32). No elevated risks were found for low (OR 0.61; 95% CI 0.20–1.86) or high use (OR 0.62; 95% CI 0.23–1.69) and early-diagnosed cases or for low use and late-diagnosed cases (OR 2.16; 95% CI 0.95–4.93). In the much

larger AHS analyses of 309 cases and 30,145 non-cases, RRs for 2,4-D calculated using Poisson regression and controlling for confounding factors were not elevated (Engel et al. 2005). The RR for wife's 2,4-D use among all wives in the cohort was 0.8 (95% CI 0.6–1.1) and for husband's 2,4-D use among wives who never used pesticides was 0.9 (95% CI 0.6–1.4). No associations were also found in analyses of farmer's wives by state (OR 0.7; 95% CI 0.6–1.0) or by menopausal status at enrollment (OR 1.2; 95% CI 0.7–2.1).

Cancer of the Nervous System. Two studies provide information regarding exposure to 2,4-D and cancer of the nervous system. A case-control study of residents (251 cases, 498 controls) from 66 counties in eastern Nebraska reported an association between increased risk of glioma and ever living or working on a farm and/or the duration of farming (OR 3.9; 95% CI 1.8–8.6) (Lee et al. 2005). However, an increased risk was found with 2,4-D exposure only when the questionnaire assessing demographics, smoking and alcohol consumption, diet, family history of cancer, complete residential and occupational history, medical history and other factors was completed by proxies (in most cases, spouses or first-degree relatives) (OR 3.3; 95% CI 1.5–7.2), but not cases themselves (OR 0.6; 95% CI 0.2–1.6). A similar study of 798 histologically confirmed primary glioma cases and 1,175 population-based controls (non-metropolitan residents of four Midwest states) reported an inverse association between use of 2,4-D and incidence of glioma (OR 0.64; 95% CI 0.47–0.88) (Yiin et al. 2012). No association was found when proxy respondents were excluded (OR 0.76; 95% CI 0.51–1.11). The limited information available does not support an association between exposure to 2,4-D and glioma.

Prostate Cancer. A few studies provide information regarding exposure to 2,4-D and prostate cancer. No association was found in the AHS (p-value for trend=0.53, adjusted for age and family history of prostate cancer) (Alavanja et al. 2003). In a much smaller study of Dutch chlorophenoxy herbicide manufacture workers, the hazard ratios (HRs) were elevated in the two factories examined (HR 2.93; 95% CI 0.61–14.5; HR 2.68; 95% CI 0.48–14.85) based on six cases among exposed workers and two among non-exposed workers in one factory and four cases among exposed workers and two among non-exposed workers in the other factory (Boers et al. 2010). A cohort study of 1,256 workers involved in the manufacture of 2,4-D in Michigan, reported a risk deficit of prostate cancers among the workers compared to Michigan white males (SIR 0.74; 95% CI 0.57– 0.94) (Burns et al. 2011). A case-control study of British Columbia farmers with potential exposure to multiple chemicals reported an elevated OR among those ever exposed to 2,4-D compared to an unexposed group (OR 2.72; 95% CI 1.12–6.57) (Band et al. 2011). Because there were only 12 exposed cases, dose-response analyses were not

performed. Significant inconsistencies between studies preclude making any statement about the possibility of hazard.

Other Cancers. A study of 1,256 male workers employed in the manufacturing of 2,4-D in Midland, Michigan, reported an excess risk of "other respiratory" cancers compared to Michigan white males (SIR 3.79; 95% CI 1.22–8.84) (Burns et al. 2011). Five cases were observed compared to 1.32 expected. The "other respiratory" category excluded cancers of the larynx, bronchus, trachea, and lung and included nasal cavity, accessory sinuses, pleura, and other sites. Four of the five cases were mesotheliomas, which the investigators noted is strongly associated with exposure to asbestos; however, the workers' detailed job histories were not available due to confidentiality agreements.

In the AHS, no association was found between ever/never use of 2,4-D among herbicide applicators and spouses and pancreatic cancer (OR 0.9; 95% CI 0.5–1.5) (Andreotti et al. 2009). In addition, ORs for pancreatic cancer showed no relation to intensity-weighted exposure to 2,4-D among applicators. ORs for never use, low-intensity exposure, and high-intensity exposure were 1.0, 0.8 (95% CI 0.4–1.6), and 0.9 (95% CI 0.5–1.7), respectively.

Data regarding cancer in animals are limited to a case-control study of malignant lymphoma in household dogs from residences where 2,4-D herbicides were applied onto lawns by the dog's owner and/or by commercial lawn care companies (Hayes et al. 1991). It seems reasonable to assume that the main route of exposure to the herbicides was by dermal contact, although it is likely that some ingestion also occurred by the dogs licking their paws. Dogs have been shown to absorb 2,4-D from lawns treated with products containing 2,4-D by measuring urinary levels of 2,4-D at various times after application of the product (Reynolds et al. 1994). The study by Hayes et al. (1991) included 491 dogs with lymphoma matched on age to 479 tumor control dogs and 466 non-tumor control dogs. Exposure was assessed by self-administered owner questionnaire and/or telephone interview. The investigators found a weak, but significant association between exposure to 2,4-D and risk of canine malignant lymphoma (OR 1.3; 95% CI, 1.04–1.67). However, an evaluation of the study by a scientific review panel found that numerous limitations in the study design, the most significant of which was exposure quantification, may have led Hayes et al. (1991) to erroneous conclusions (Carlo et al. 1992). The review panel noted, for example, that when separate analyses were conducted for commercial lawn treatment only, owner application of 2,4-D only, and both groups combined, none of the associations showed statistical significance. It was also noted that no clear dose-response trends were observed for number of commercial lawn chemical applications per year, but a positive increasing lymphoma risk trend was reported with annual number of

owner applications of 2,4-D. In a later publication, Hayes et al. (1995) addressed many of the criticisms raised regarding the original study and clarified the conclusions by noting that the small reported association was in the range that could be easily explained by bias or confounding. They also stated that the results should be interpreted with caution given the relatively low exposure levels and the problems related to exposure assessment. Kaneene and Miller (1999) reanalyzed the data using a more restrictive exposure definition and found that the numbers of dogs in the various exposure categories were substantially different than the numbers reached in the original study. Based on this redistribution of dogs, Kaneene and Miller (1999) could not confirm a dose-response relationship between 2,4-D use and malignant lymphoma.

The EPA has assigned 2,4-D to carcinogenicity Group D, "not classifiable as to human carcinogenicity" (EPA 2005a). The International Agency for Research on Cancer (IARC) recently classified 2,4-D as possibly carcinogenic to humans (Group 2B) based on inadequate evidence in animals and limited evidence in experimental animals (IARC 2016; Loomis et al. 2015).

3.3 GENOTOXICITY

2,4-D has shown mixed results for genotoxic activity in *in vivo* and *in vitro* tests with organisms ranging from bacteria to humans. Tables 3-4 and 3-5 present a cross-section of some of the genotoxicity data that are available for 2,4-D in *in vivo* and *in vitro* test systems.

In vivo Exposure Studies. Results from human *in vivo* exposure genotoxicity studies are mixed (Table 3-4). The association of occupational pesticide use and relative telomere length (shorter telomere length has been associated with increased risk of cancer) was investigated in a cohort of 1,234 cancer-free white male pesticide applicators in the AHS (Hou et al. 2013). Exposure to 2,4-D, as assessed through questionnaires, was significantly associated with a decrease in relative telomere length ($p=0.004$) after adjusting for age at buccal cell collection, state of residence, license type, use of chewing tobacco, and total pesticide-application days. Similar results were reported in a subsequent evaluation of leukocyte DNA from 568 cancer-free males in the AHS (p-trend=0.001) (Andreotti et al. 2015). Increased chromosomal aberrations in lymphocytes were reported in another occupational study that investigated the effect of 2,4-D and 2,4,5-T production on plant workers (Kaioumova and Khabutdinova 1998). However, because of limitations including the relatively small sample of only 19 participants, the apparent lack of control for confounders, suspected mixed exposure, and no measures of exposure, the results should be interpreted with caution. Negative results for chromosomal aberrations or micronuclei

Table 3-4. Genotoxicity of 2,4-D *In Vivo*

Species (test system)	End point	Results	Reference
Human (occupational exposure/buccal cells)	Telomere length	+	Hou et al. 2013
Human (occupational exposure/peripheral blood leukocytes)	Telomere length	+	Andreotti et al. 2015
Human (occupational exposure/lymphocyte culture, urine)	Chromosome aberrations	–	Garry et al. 2001
Human (occupational exposure; peripheral blood lymphocytes)	Chromosome aberrations	+	Kaioumova and Khabutdinova 1998
Human (occupational exposure; peripheral lymphyocytes)	Chromosome aberrations	–	Mustonen et al. 1986
Human (occupational exposure/blood and urine)	Micronuclei frequency	–	Figgs et al. 2000
Human (occupational exposure/blood and urine)	Lymphocyte proliferation	+	Figgs et al. 2000
Human (occupational exposure/peripheral lymphocytes)	Micronuclei frequency	–	Holland et al. 2002
Mouse (host-mediated assay using *Salmonella typhimurium and Saccharomyces cerevisiae as indicators)*	Mutation (host-mediated assay)	–	Zetterberg et al. 1977[a]
Mouse (gestational exposure, fetal deaths)	Mutation; dominant lethal assay	–	Epstein et al. 1972
Mouse (bone marrow, spermatocyte cells)	Chromosome aberrations; sperm-head abnormalities	+	Amer and Aly 2001
Mouse (bone marrow)	Chromosome aberrations	+	Venkov et al. 2000
Mouse (bone marrow)	Chromosome aberrations	–	Yilmaz and Yuksel 2005
Mouse (bone marrow and spermatogonial cells)	Sister chromatid exchange	+	Madrigal-Bujaidar et al. 2001
Mouse (hair follicle)	Hair follicle nuclear aberration test	+	Schop et al. 1990
Mouse (bone marrow)	Micronucleus test	–	Schop et al. 1990
Mouse (bone marrow)	Micronucleus test	–	Charles et al. 1999b
Rat (blood lymphocytes)	Sister chromatid exchange	–	Linnainmaa 1984
Rat (lymphocytes)	Sister chromatid exchange	–	Mustonen et al. 1989
Rat (primary hepatocytes)	Unscheduled DNA synthesis	–	Charles et al. 1999a
Rat (primary hepatocytes, white blood cells)	DNA damage	–	Kitchin and Brown 1988
Chinese Hamster (bone marrow cells)	Sister chromatid exchange	–	Linnainmaa 1984

Table 3-4. Genotoxicity of 2,4-D *In Vivo*

Species (test system)	End point	Results	Reference
Non-mammalian cells:			
Drosophila melanogaster	Somatic mutation and recombination (wing spot test)	(+)	Kaya et al. 1999
D. melanogaster	Somatic mutation (wing spot test)	+	Tripathy et al. 1993
D. melanogaster	Sex-linked recessive mutation	+	Tripathy et al. 1993
D. melanogaster	Sex-linked recessive mutation	(+)	Magnusson et al. 1977
D. melanogaster	Sex-linked recessive mutation	+	Rasmuson and Svahlin 1978
D. melanogaster	Sex-linked recessive mutation	(+)	Vogel and Chandler 1974

[a]Study conducted using 2,4-D sodium salt.

– = negative result; + = positive result; (+) = weak positive result; 2,4-D = 2,4-dichlorophenoxyacetic acid

Table 3-5. Genotoxicity of 2,4-D *In Vitro*

Species (test system)	End point	Results: With activation	Results: Without activation	Reference
Prokaryotic organisms:				
Salmonella typhimurium TA98, TA100, TA1535, TA1535, TA1537, TA1538 (Ames test)	Gene mutation	–	–	Charles et al. 1999a
S. typhimurium TA 98, TA100	Gene mutation	–	–	Kubo et al. 2002
S. typhimurium TA97, TA98, TA100, TA102; *Escherichia coli*	Gene mutation/SOS chromatid test	–	–	Mersch-Sundermann et al. 1994
S. typhimurium	Mutation (host mediated assay)	No data	–	Styles 1973
S. typhimurium TA1530, TA1535, TA1531, TA1583	Mutation	No data	–	Zetterberg et al. 1977[a]
S. typhimurium. uvrB, rec; E. coli; Bacillus subtilis rec	DNA damage	No data	+	Garrett et al. 1986
E. coli	Mutation (modifed SOS microplate assay)	No data	–	Venkat et al. 1995
Saccharomyces cerevisiae strain *D7ts1*	Mitotic gene conversion; reverse mutation	No data	+	Venkov et al. 2000
S. cerevisiae strains D4, D5	Mitotic gene conversion; recombination	No data	+	Zetterberg et al. 1977[a]
S. cerevisiae strain RAD 18	Mitotic gene conversion; recombination	No data	+	Zetterberg 1978
Eukaryotic organisms:				
Human fibroblasts	Mutation (colony forming ability, single strand breaks)	No data	–	Clausen et al. 1990
Human fibroblasts	Mutation (colony forming ability, single strand breaks)	No data	+	Clausen et al. 1990[b]
Human lymphocytes	Sister chromatid exchange	No data	+	Korte and Jalal 1982
Human lymphocytes (whole blood and leukocyte cultures)	Sister chromatid exchange	No data	+	Soloneski et al. 2007
Human lymphocytes	Sister chromatid exchange	No data	+	Turkula and Jalal 1985
Human lymphocytes	Chromosome aberrations	–	–	Mustonen et al. 1986
Human lymphoma and leukemia cells	Chromosome aberrations	No data	+	Venkov et al. 2000

Table 3-5. Genotoxicity of 2,4-D *In Vitro*

Species (test system)	End point	Results		Reference
		With activation	Without activation	
Human lymphocytes	Chromosome aberrations; micronucleus assay	+	+	Zeljezic and Garaj-Vrhovac 2004
Human lymphocytes	DNA damage	No data	+	Sandal and Yilmaz 2011
Chinese hamster (V79 cell culture)	Mutation	No data	+	Ahmed et al. 1977
Chinese hamster (CHO cells)	Chromosome aberrations	+	–	Galloway et al. 1987
Chinese hamster (CHO cells)	Sister chromatid exchange	–	+	Galloway et al. 1987
Chinese hamster (CHO cells)	Sister chromatid exchange	No data	+	González et al. 2005
Chinese hamster (CHO cells)	Sister chromatid exchange	–	–	Linnainmaa 1984
Chinese hamster (CHO cells)	DNA damage	No data	+	González et al. 2005
Rat (primary hepatocytes)	Unscheduled DNA synthesis	No data	–	Charles et al. 1999a
Syrian Golden Hamster embryo (SHE cells)	Morphological cell transformation, DNA damage	No data	+	Maire et al. 2007
Syrian Golden Hamster embryo (SHE cells)	Morphological cell transformation	No data	–	Mikalsen et al. 1990

[a]Study conducted using 2,4-D-sodium salt.
[b]Study conducted using 2,4-D-ammonium salt.

– = negative result; + = positive result; (+) = weakly positive; 2,4-D = 2,4-dichlorophenoxyacetic acid; CHO = Chinese hamster ovary; DNA = deoxyribonucleic acid

were found in additional occupational exposure studies (Figgs et al. 2000; Garry et al. 2001; Holland et al. 2002; Mustonen et al. 1986). Lymphocyte proliferation (replicative index) and micronuclei frequency was determined in urine specimens of 12 herbicide spraying applicators (Figgs et al. 2000). Proliferation index increased in the exposed group after first exposure ($p=0.016$) and was also greater among the exposed than among a control group of non-applicators ($p=0.046$). Urinary 2,4-D was associated with increased proliferation index after spraying; however, no statistically significant dose-response was observed. In a study by Garry et al. (2001), urinary levels of 2,4-D were measured in 24 herbicide applicators and 15 minimally exposed controls. With this limited sample size, urinary 2,4-D levels were not statistically correlated with frequency of chromosomal aberrations, and the amount of 2,4-D applied had no direct effect on urinary 2,4-D. Garry et al. (2001) noted that due to the relatively small sample size, the results need to be interpreted with caution. In another small study of only 19 forest workers exposed to 2,4-D and 15 controls, there was no increase in the incidence of chromosomal aberrations in the lymphocytes of herbicide sprayers, as measured in blood samples taken after the spraying season (Mustonen et al. 1986). There was also no association between urinary 2,4-D and length of exposure in this study (9–11 days). The small number of subjects studied limits the interpretation of the results of this study.

In animal studies, oral exposure to 2,4-D has been found to cause chromosomal aberrations, sister chromatid exchanges, and sperm-head abnormalities in somatic and germ cells of mice (Amer and Aly 2001; Madrigal-Bujaidar et al. 2001; Venkov et al. 2000). Acute dermal exposure to 2,4-D increased the incidence of hair follicle nuclear aberrations in mice (Schop et al. 1990). Other studies reported negative findings for chromosomal aberrations and sister chromatid exchanges (SCEs) in bone marrow and lymphocytes following oral exposure in mice, rats, and Chinese hamsters (Linnainmaa 1984; Mustonen et al. 1989; Yilmaz and Yuksel 2005). Negative results were also reported in a dominant lethal mutation assay in mice (Epstein et al. 1972), in two mice micronucleus tests (Charles et al. 1999b; Schop et al. 1990), and in assays for unscheduled DNA synthesis and DNA damage in primary hepatocytes and white blood cells of rats following oral exposures (Charles et al. 1999a; Kitchin and Brown 1988). A host-mediated assay in mice was negative using *Salmonella typhimurium and Saccharomyces cerevisiae* as indicators for mutation following oral exposure to 2,4-D sodium salt (Zetterberg et al. 1977). *In vivo* 2,4-D exposure produced weakly positive results in a wing spot test (Kaya et al. 1999) and in sex-linked recessive mutation tests (Magnusson et al. 1997; Rasmuson and Svahlin 1978; Volgel and Chandler 1974) in *Drosophila melanogaster*. Positive results in these two tests in *Drosophila* were reported by Tripathy et al. (1993). It was suggested that binding of 2,4-D to DNA may induce conformational changes to the DNA molecule (Ahmadi and Bakhshandeh 2009).

In vitro Exposure Studies. As summarized in Table 3-5, 2,4-D was not mutagenic in *S. typhimurium* or *Escherichia coli* (Charles et al. 1999a; Kubo et al. 2002; Mersch-Sundermann et al. 1994; Venkat et al. 1995) and 2,4-D sodium salt was not mutagenic in *S. typhimurium* (Zetterberg et al. 1977). Negative results were also reported in an *in vitro* host-mediated assay in mice using *S. typhimurium* as an indicator for 2,4-D mutation (Styles 1973). In contrast, positive results were reported for DNA damage in *S. typhimurium, E. coli,* and *Bacillus subtilis* (Garrett et al. 1986). 2,4-D and the 2,4-D sodium salt also produced positive results for mitotic gene conversion and reverse mutations in *S. cerevisiae* (Venkov et al. 2000; Zetterberg et al. 1977, 1978).

A number of human cell lines have been tested with 2,4-D giving positive results without metabolic activation, resulting in DNA damage, increased micronuclei, chromosomal aberrations, and SCEs (Korte and Jalah 1982; Sandal and Yilmaz 2011; Soloneski et al. 2007; Turkula and Jalal 1985; Venkov et al. 2000; Zeljezic and Garaj-Vrhovac 2004). In one study, the 2,4-D ammonium salt produced mutations in human fibroblasts; however, results for 2,4-D acid were negative in the same assay (Clausen et al. 1990). Negative results were also reported for chromosomal aberrations following exposure of human lymphocytes to 2,4-D (Mustonen et al. 1986). In this study, positive results for chromosomal aberrations were reported in the absence of metabolic activation using commercial 2,4-D, but negative results were obtained when purified 2,4-D was tested. The investigators suggested the different results may have been due to the commercial formulation containing an unidentified chlorophenol contaminant.

In vitro studies with other mammalian cells have demonstrated mainly positive results for mutation, chromosomal aberrations, sister chromatid exchange (SCEs), DNA damage, and morphological cell transformation in Chinese and Syrian hamster cell lines (Ahmed et al. 1977; Galloway et al. 1987; González et al. 2005; Maire et al. 2007). Negative results were reported in other studies for SCEs in Chinese hamster ovary cells (Linnainmaa 1984), unscheduled DNA synthesis in primary rat hepatocytes (Charles et al.1999a), and morphological cell transformation in Syrian golden hamster cells (Mikalsen et al. 1990).

In summary, although results of genotoxicity studies in humans, animals, and *in vitro* studies have been mixed, the fact that some studies have reported positive results supports a biological plausibility of effects occurring as a result of exposure to 2,4-D and cannot be discounted.

3.4 TOXICOKINETICS

2,4-D is rapidly and almost completely absorbed from the gastrointestinal tract in humans and animals, but dermal absorption is relatively low (<10% of an applied dose in humans). 2,4-D distributes widely in tissues following oral exposure, does not accumulate in tissues, is subject to limited metabolism, and is eliminated via the kidneys by a mechanism that involves a saturable carrier protein. Studies in humans have estimated elimination half-lives in urine of <2 days following single oral or dermal doses of 2,4-D. In animals, 2,4-D can be transferred to fetal tissues and to offspring through maternal milk, although this has not been definitively proven in humans. The toxicokinetics of 2,4-D is species- and sex-dependent largely due to differences in renal clearance of 2,4-D. This differential capacity for excreting 2,4-D plays an important role in the susceptibility to 2,4-D-induced effects between species.

3.4.1 Absorption

3.4.1.1 Inhalation Exposure

No studies were located regarding absorption of 2,4-D following inhalation exposure.

3.4.1.2 Oral Exposure

Evidence of gastrointestinal absorption of 2,4-D in humans comes from analysis of 2,4-D in tissues and fluids from cases of intentional or accidental ingestion of commercial products containing 2,4-D that resulted in death and from studies with volunteers. Quantitative data are available from the latter studies.

Results from studies in volunteers have shown that oral absorption of 2,4-D in humans is rapid and virtually complete. For example, oral administration of a single dose of 5 mg/kg 2,4-D in a gelatin capsule to six male volunteers resulted in a significant amount of the compound in plasma 1 hour after dosing and in a maximum of approximately 30 µg/mL 7–24 hours after dosing (Kohli et al. 1974). Assuming first rates of absorption and clearance, the investigators estimated a plasma half-life of 33 hours. A similar study in which five male volunteers were administered 5 mg/kg analytical-grade 2,4-D reported that plasma levels achieved a maximum of 10–30 µg/g approximately 6 hours after dosing (Sauerhoff et al. 1977). Elimination from plasma appeared to follow a one-compartment model for two subjects and a one- or two-compartment model for the third subject. Two subjects were not modeled. The volumes of distribution for the former were 238 and 294 mL/kg, and 83 and 218 mL/kg for the third subject if a two-compartment model was assumed; these data suggested relatively limited distribution to

tissues. The pooled half-life value for clearance of 2,4-D from plasma was 11.6 hours. Based on recovery data, it was estimated that absorption ranged from 87.6 to 106.3% of the administered dose.

Oral absorption in animals is fast and complete, particularly at relatively low doses (≤50 mg/kg), as assessed by early detection of 2,4-D in tissues and almost complete recovery of the dose in urine (i.e., Khanna and Fang 1966). Studies in animals have also shown sex differences as well as species differences in disposition of orally absorbed 2,4-D. For example, analysis of plasma concentrations of 2,4-D in rats following oral administration of a 5 mg/kg dose showed no difference in absorption rates between males and females. In a study in dogs and rats administered a single oral dose of 5 or 50 mg/kg ^{14}C-2,4-D, rats eliminated radioactivity from plasma significantly faster than dogs (van Ravenzwaay et al. 2003). Approximate elimination half-lives were 1.3–3.4 hours in rats and 99–134 hours in dogs following the low- and high-dose, respectively. This resulted in areas under the curve ($AUC_{[0-\infty]}$) significantly higher in dogs than in the rats. In addition, over the monitoring period of 120 hours, elimination of radioactivity from plasma was complete in rats, but not in dogs.

3.4.1.3 Dermal Exposure

Dermal absorption of 2,4-D in humans is low compared to oral absorption. Male volunteers that received a topical application of 4 μg/cm^2 of 2,4-D in acetone on the ventral forearm excreted only 5.8% of the applied dose in the urine over a 5-day monitoring period (Feldmann and Maibach 1974). The application site was not protected and the subjects were asked not to wash the site for 24 hours. These results are consistent with those from a similar study in male volunteers that reported that 4.5% of an applied dose of 10 mg 2,4-D in acetone/water over a 9 cm^2 area on the dorsum of the hand was absorbed over a 144-hour period (Harris and Solomon 1992). Using data from Feldmann and Maibach (1974) in an exponential saturation model with lag time, Thongsinthusak et al. (1999) estimated dermal absorption of 2,4-D in humans to be 21.2–21.7% of the applied dose. In a review of the literature, however, it was noted that because the results of Harris and Solomon (1992) indicated that excretion of 2,4-D was essentially complete by 144 hours, using models much beyond 120 hours will over predict absorption (Ross et al. 2005), so the results of Thongsinthusak et al. (1999) are not reliable.

Based on recovery of 2,4-D in the urine, a comparative study showed that rabbits absorbed a higher percentage (36% of the dose) of the applied dose than monkeys and that absorption rate can vary with the application site (Moody et al. 1990). Monkeys absorbed almost twice the amount of 2,4-D when the compound was applied on the forehead (29% of the dose) than when applied on the forearm (15% of the

dose). Another study in monkeys reported an absorption rate of 8.6% of the dose when 2,4-D in acetone was applied on the abdomen of the animals (Wester et al. 1996). Application of 2,4-D in soil onto a 12-cm^2 area of abdominal skin lightly clipped resulted in absorption of 9.8% of the dose when the soil load was 1 mg/cm^2 and 15.9% when the soil load was 40 mg/cm^2. Because the dose of 2,4-D applied was the same with both soil loads, the results showed that, under the conditions of the study, dermal absorption from soil was not significantly affected by soil load (Wester et al. 1996). However, a study with human skin *in vitro* in which the concentration of 2,4-D in soil was 5 ppm (5 mg 2,4-D/kg soil) reported that dermal absorption of 2,4-D was dependent on both soil load and also on the type of soil (Duff and Kissel 1996).

In a comparative study in which rats and guinea pigs were applied ^{14}C-2,4-D onto the skin, rats and guinea pigs absorbed a total of 49% and 40%, respectively, of the applied dose over a 14-day monitoring period (Moody et al. 1994). The value estimated for the rat in this *in vivo* study was consistent with a 40% absorption estimated in a dermatomed skin preparation *in vitro*, but not so for the guinea pig in which only 14% of a 2,4-D dose was absorbed through a skin preparation *in vitro*. For comparison purposes, 19% of a dose of 2,4-D in acetone was absorbed through human skin *in vitro* and 14% through pig skin *in vitro* (Moody et al. 1994). Approximately 2% of 2,4-D in soil was absorbed through human skin *in vitro* (Wester et al. 1996). However, when using acetone as a vehicle, 19% of an applied dose of 2,4-D was absorbed (Moody et al. 1994).

In mice, approximately 7% of a dose of 1 mg/kg of ^{14}C-2,4-D in acetone penetrated the body (disappeared from the covered site of application) in 1 hour and about 21% in 24 hours (Grissom et al. 1985).

A series of studies by Brand and coworkers (Brand et al. 2002, 2003, 2004, 2007a, 2007b) examined factors that can influence the dermal absorption of 2,4-D in animal models. Using hairless mice skin *in vitro*, the investigators reported that six out of nine commercially available sunscreens significantly increased the total penetration of 2,4-D through the skin over a 24-hour period (Brand et al. 2002). Total penetration of 2,4-D ranged from 39.1% for no sunscreen used to 81.0% for the sunscreen that facilitated penetration the most. Subsequent studies showed that ultraviolet (UV) absorbers in sunscreens significantly enhanced the transdermal absorption of 2,4-D (Brand et al. 2003; Pont et al. 2004). The investigators also showed that dietary exposure of rats to ethanol for 6–8 weeks resulted in increased penetration of 2,4-D through the rat skin in an *in vitro* diffusion system, most likely due to altering the properties of the dermal barrier, possibly by inducing changes in lipid peroxidation and increasing transepidermal water loss (Brand et al. 2004, 2007a). Results from an additional study showed that the

combination of sunscreen use and ethanol ingestion enhanced penetration of 2,4-D in rats' skin in an additive manner (Brand et al. 2007b).

3.4.1.4 Other Routes of Exposure

Analysis of plasma from rats following an intravenous injection of 5 mg/kg 2,4-D showed a significantly smaller volume of distribution in females (50.2 mL) than in males (80.6 mL), consistent with significantly higher plasma concentration of 2,4-D (Griffin et al. 1997a). In addition, clearance (mL/minute) was about 10-fold lower in females than in males, whereas elimination half-lives from plasma were significantly higher in females.

3.4.2 Distribution

3.4.2.1 Inhalation Exposure

No information was located regarding distribution of 2,4-D following inhalation exposure of humans or animals.

3.4.2.2 Oral Exposure

Distribution data for 2,4-D following oral ingestion by humans are available in case reports that resulted in death; the results showed wide distribution in tissues. For example, reports by Dudley and Thapar (1972), Nielsen et al. (1965), Osterloh et al. (1983), and Keller et al. (1994) showed measurable amounts of 2,4-D in all organs that were examined, including the brain, liver, kidney, spleen, muscle, body fat, pancreas, heart, and lungs.

Studies in animals have shown that 2,4-D is widely distributed in tissues after oral dosing. In a study in rats, some 2,4-D-derived radioactivity was detected in all 12 tissues examined as early as 1 hour after gavage dosing (Khanna and Fang 1966). Rats were given approximately 3 or 240 mg/kg 2,4-D. With the low dose, peak concentration in tissues was achieved 6–8 hours after dosing. Elimination was fast (half-life 0.58 hours), with no detectable radioactivity in tissues 24 hours after dosing. Aside from the stomach, the kidneys had the highest amount of radioactivity and the brain had the least; no radioactivity could be detected in the brain within the first 4 hours after dosing. In high-dose rats, peak concentrations in tissues occurred 8 hours after dosing and could still be detected in tissues 41 hours after dosing. Elimination half-lives ranged from 3 to 3.5 hours; the brain had the lowest amount of label at all times and the kidneys

had the highest. Examination of the intracellular distribution of 2,4-D in the nuclear, mitochondrial, microsomal, and soluble fractions of the kidneys, liver, spleen, brain, heart, and lungs showed that all fractions contained significant radioactivity. Regardless of the dose, most radiolabel was found in the soluble and nuclear fractions, while the microsomal and mitochondrial fractions only contained 1.4–6.7% of the total radioactivity. Because the radioactivity in the soluble fraction from all tissues could easily be extracted with ether, Khanna and Fang (1966) suggested that the 2,4-D molecule in the soluble fraction was not protein- or peptide-bound.

A comparative study in rats, mice, and hamsters of both sexes showed that ^{14}C-2,4-D-derived radioactivity was widely distributed in tissues following a single oral dose (5 or 200 mg/kg) of 2,4-D, but differences between sexes were apparent in rats and hamsters (Griffin et al. 1997a). In general, over a 72-hour monitoring period, liver and kidneys appeared to have the most radioactivity at early time points (2–8 hours); skin and fat showed relatively high amounts of radioactivity throughout the monitoring period in animals given the high dose of 2,4-D. Tissues levels of radioactivity were consistently higher in female rats than in male rats, although the differences were not always statistically different. In hamsters, tissue levels of radioactivity were more often than not higher in males than in females. No clear differences in disposition of radioactivity were established between male and female mice.

A study in rats showed that postnatal dietary maternal exposure to 2,4-D can result in transfer of 2,4-D to the offspring via the milk (Stürtz et al. 2006). Over a dose range of 15–70 mg/kg, the concentrations of 2,4-D in dams' serum, milk, and 16-day-old pups' serum were dose-dependent, but were significantly lower in pups' serum than in maternal media. The study also showed that maternal exposure to 2,4-D altered the contents of lipids (30% decreased at 25 mg 2,4-D/kg/day) and of some proteins in the milk. More recently, Saghir et al. (2013) also demonstrated excretion of 2,4-D in rat's milk following perinatal exposure to 2,4-D via the diet. On lactation day 4, the concentration of 2,4-D in milk was 1.7–6.3 times lower than the concentration in the dams' plasma. The ratio was reduced to 1.5–2.5 times lower on lactation day 14 due to an approximate doubling of the dams' intake of 2,4-D in the 10-day interval. The concentration of 2,4-D in pups' plasma also increased from PND 4 to 10. Over the range of dietary concentrations tested (10–1,600 ppm 2,4-D), the ratios of pups' plasma 2,4-D/maternal plasma 2,4-D increased greatly on PND 14 relative to PND 4.

3.4.2.3 Dermal Exposure

No information was located regarding distribution of 2,4-D following dermal exposure of humans or animals. However, since dermal absorption occurs, it is reasonable to assume that 2,4-D will distribute in a manner similar to that reported in oral animal studies.

3.4.2.4 Other Routes of Exposure

In adult male rats, subcutaneous administration of a dose of 250 mg/kg 2,4-D followed by intravenous dosing of radiolabeled 2,4-D resulted in most of the radiolabel in the plasma, kidneys, and liver about 2 hours after dosing (Elo and Ylitalo 1979). Somewhat lower amounts were reported in the lungs and heart, and significantly lower amounts were found in the brain, muscle, testes, and cerebral spinal fluid. In a study that only evaluated brain distribution, subcutaneous administration of 300 mg/kg 2,4-D (half the LD_{50}) followed by intravenous radioactive 2,4-D resulted in radioactivity widely distributed in various brain areas (cerebral cortex, striatum, medulla oblongata, cerebellum, and midbrain brain, including hippocampus, hypothalamus, and thalamus) without any one area showing preferential accumulation of radioactivity (Tyynelä et al. 1990). In adult rabbits, administration of a single intraperitoneal low dose of ^{14}C-2,4-D resulted in wide distribution of radioactivity throughout the brain 2 hours after dosing, and ranged from 2.8% of plasma in the hypothalamus to 4.58% in the brainstem (Kim et al. 1988).

Intravenously injected 2,4-D to pregnant mice tended to accumulate in the visceral yolk sac and after passing to the fetus, was eliminated from all tissues within 24 hours (Lindquist and Ullberg 1971). Another study in pregnant mice given an intraperitoneal injection of ^{14}C-2,4-D on GD 17 showed that 3 hours after dosing, radioactivity was distributed in various brain regions and ranged from a low of 2.8% of that of plasma in the caudate nucleus to 4.6% in the brainstem (Kim et al. 1988). Fetal brain as a whole contained 5.8% of the amount in plasma, suggesting that the brain barrier forms early in fetal life. Intravenous injection of ^{14}C-2,4-D to pregnant rabbits on GDs 28–30 resulted in rapid transfer of radioactivity to fetal plasma and brain (Sandberg et al. 1996). Peak concentrations of radiolabel were achieved in fetal plasma approximately 30 minutes after injection and remained relatively constant for the remainder of the 2-hour sampling period. Except for radiolabel in plasma, maternal kidneys and uterus showed the highest tissue AUCs. In maternal brain, lateral and ventricular choroid plexus had the highest concentration of radioactivity (about 10 times higher than any other brain region). Fetal brain had the lowest concentration of label of any maternal or fetal organ sampled. However, the concentration in fetal brain tissue was 7% of that in fetal plasma compared to 2% of that in maternal plasma, suggesting possible increased vulnerability of the fetus. In general, maternal and fetal tissue AUCs increased

proportionally as the dose of 2,4-D increased from 1 to 10 mg/kg; however, in fetal tissues, it also increased 10-fold when the maternal dose increased from 10 to 40 mg/kg. The investigators suggested that because only unbound compound was available for placental transfer, the greater increase in fetal AUCs suggested saturation of maternal 2,4-D plasma protein binding (Sandberg et al. 1996).

Transfer of 2,4-D to the offspring was also observed in rats following intraperitoneal injections to nursing dams every other day up to postnatal day (PND) 16 (Stürtz et al. 2000). Transfer to 2,4-D was evident already in 4-day-old pups. In general, 2,4-D residues in pups' stomach contents, blood, kidney, and brain were dose- and exposure-time-dependent. The stomach content (milk) and the kidneys always contained the highest concentrations of 2,4-D. Levels of 2,4-D in kidneys in 8-day-old offspring from high-dose dams (100 mg/kg) increased 6-fold compared to 4-day-old pups. Pups' brain always had the lowest concentration of 2,4-D, which varied 10-fold between low-dose (50 mg/kg) 4-day-old pups and high-dose 16-day-old pups. The latter gained significantly less weight than controls, which the investigators attributed to diminished milk intake and/or a direct toxic effect of 2,4-D. Unlike Stürtz et al. (2006), these investigators discounted the quality of milk as a reason for less weight gain.

3.4.3 Metabolism

Studies in humans and animals have shown that 2,4-D undergoes limited metabolism in the body based on identification and quantification of products in the urine. For example, in a group of six male volunteers, only unchanged 2,4-D was detected in urine samples over a 1-week period after receiving a single oral dose of 5 mg/kg 2,4-D in a gelatin capsule (Kohli et al. 1974). In a similar study, analysis of urine samples from five volunteers following ingestion of 5 mg/kg 2,4-D showed mostly unchanged parent compound (mean 82.3% of the administered dose) with smaller amounts (mean 12% of the dose) excreted as a 2,4-D conjugate over a 6-day period (Sauerhoff et al. 1977).

Studies in animals have shown that, depending on the species, 2,4-D does not undergo metabolism, or if it does, as in dogs, it undergoes phase II metabolism to form conjugates that are excreted mainly in the urine; the biliary system plays only a minor role (Griffin et al. 1997b). Griffin et al. (1997a) studied the metabolism of 2,4-D in rats, mice, and hamsters and reported qualitative and quantitative differences in metabolite profiles between species, but not between sexes. Following administration of an oral dose of 5 or 200 mg/kg ^{14}C-2,4-D the parent compound was the major urinary metabolite in the three species. A glycine conjugate was identified in the urine from mice and hamsters, a taurine conjugate was present in the urine from mice and male hamsters, and a glucuronide was detected only in urine from hamsters.

Male mice metabolized 2,4-D to the glycine conjugate to a greater extent than female mice. A more recent comparative study in rats and dogs administered a single oral dose of 5 or 50 mg/kg ^{14}C-2,4-D reported that 2,4-D was excreted unmetabolized in the urine as parent compound (van Ravenzwaay et al. 2003). In dogs, however, 2,4-D formed taurine, serine, glycine, glutamic acid, cysteine, sulfate, and glucuronide conjugates, which were excreted in the urine; dog plasma only contained unchanged 2,4-D. In general, although conjugation is minimal, it favors elimination in the urine. Figure 3-3 shows a proposed metabolic pathway for 2,4-D in dogs.

3.4.4 Elimination and Excretion

3.4.4.1 Inhalation Exposure

No data were located regarding elimination of 2,4-D in humans or in animals following inhalation exposure. However, 2,4-D has been measured in the urine of workers who experienced multi-route exposure, including inhalation (see Section 3.8.1 Biomarkers Used to Identify or Quantify Exposure to 2,4-D).

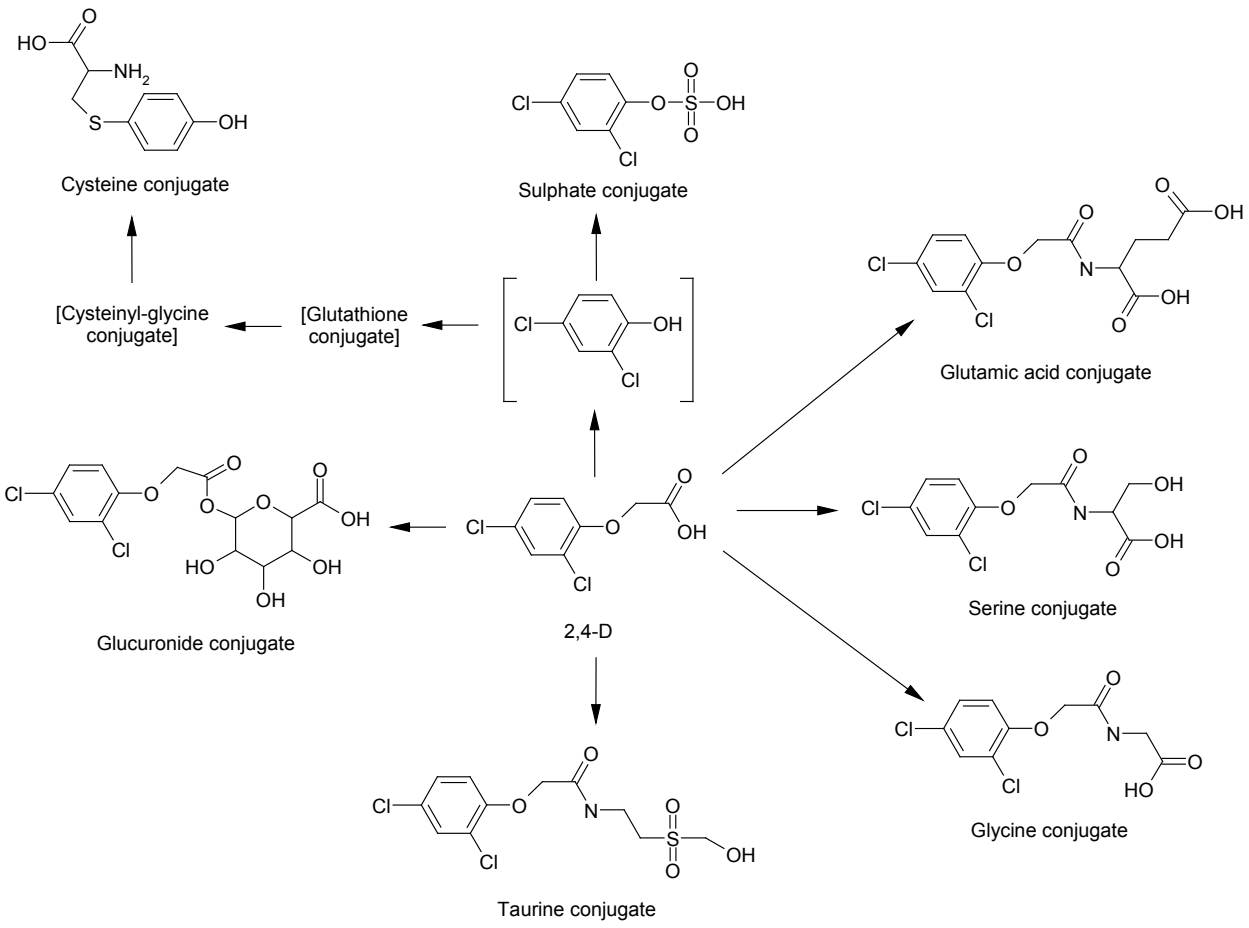
3.4.4.2 Oral Exposure

In six healthy male volunteers administered a gelatin capsule with 5 mg/kg 2,4-D, unchanged compound was detected in the urine as early as 2 hours after ingestion; >75% of the parent compound was excreted in the urine in 96 hours (Kohli et al. 1974). A similar study with volunteers reported that most of a single oral dose of 5 mg/kg 2,4-D was excreted unchanged in the urine within 3 days of dosing (Sauerhoff et al. 1977). Over a 6-day period after dosing, recovery of the administered dose was almost complete. Half-life elimination from urine ranged from 10.2 to 28.5 hours. The estimated fraction of the dose eliminated in the urine as free 2,4-D over the 6-day period ranged from 47.8 to 96.5%.

Studies in animals show that 2,4-D is eliminated mainly in the urine as unchanged compound or as conjugate, as it occurs in dogs.

In urine from rats collected every 10 hours after gavage administration of a single dose of 2.6 mg/kg 2,4-D as the sodium salt by gavage in water, peak concentration of 2,4-D occurred in the 20-hour spot sample (Knopp and Schiller 1992). Gradual decline occurred over the next 10 hours, and by 40 hours after dosing, approximately 90% of the administered dose had been accounted for in the urine. In an earlier study in rats administered doses of approximately 3–30 mg/kg ^{14}C-2,4-D by gavage, excretion of

Figure 3-3. Proposed Metabolic Pathway of 2,4-D in Dogs



2,4-D = 2,4-dichlorophenoxyacetic acid

Source: Van Ravenzwaay et al. 2003

2,4-D was virtually complete within 48 hours of dosing and 93–96% of the dose was excreted in the first 24 hours (Khanna and Fang 1966). Almost all of the radioactivity corresponded to parent compound and was excreted in the urine; no radioactivity could be detected in expired air. Administration of higher doses (~60–300 mg/kg) resulted in a linear decrease in recovery of radiolabel in urine and feces and increased amounts were recovered in the second 24 hours after dosing. Excretion of the higher dose was still incomplete 144 hours after dosing.

In a comparative study in rats, mice, and hamsters administered a single dose of 5 or 200 mg/kg ^{14}C-2,4-D, urine was the main route of elimination of radiolabel in the three species (Griffin et al. 1997a). In rats, <4% of the administered radioactivity appeared in the feces during the 72-hour monitoring period. No 2,4-D metabolites were detected in the urine or feces from rats. Mice excreted 10–24% of administered radioactivity in the feces, and of this, 13.3% was the taurine conjugate. Hamsters excreted 6–16% of the administered radioactivity in the feces and all of it was unchanged 2,4-D. In the three species, expired air contained <1% of the administered radioactivity. In a similar study in rats and dogs administered 5 or 50 mg/kg ^{14}C-2,4-D, irrespective of the dose, rats excreted almost all of the administered radioactivity in the urine, and excretion was virtually complete 24 hours after dosing (van Ravenzwaay et al. 2003). Dogs metabolized 2,4-D (Figure 3-3). Low-dose dogs excreted approximately 38% of the dose in the urine and 10–13% in the feces over the 120-hour monitoring period. High-dose dogs excreted about equal amounts of the dose (20–25%) in the urine and feces. Excretion was not complete in dogs after the 120-hour sampling time. No significant differences regarding rates or routes of excretion between male and female animals were observed.

3.4.4.3 Dermal Exposure

In volunteers applied a dermal dose of 4 µg/cm^2 2,4-D in acetone, most of the absorbed dose was eliminated in the urine within 72 hours of dosing (Feldmann and Maibach 1974). In a similar study, subjects applied a dose of 10 mg of 2,4-D in acetone over a 9 cm^2 area excreted most of the absorbed dose in 96 hours; an average of 84.8% of the applied dose was recovered in the urine in 96 hours. The approximate mean urinary excretion half-life was 39.5 hours (Harris and Solomon 1992).

Application of an aqueous solution of 2.6 mg/kg 2,4-D sodium salt to the shaved back of rats resulted in significantly lower urinary concentration of 2,4-D than when the dose was administered orally (Knopp and Schiller 1992). Peak urinary concentration of 2,4-D occurred at about 40 hours after dosing and declined gradually thereafter. As a percentage of the applied dose, 2,4-D in the urine increased steadily

over a 116-hour period after dosing, reaching a cumulative maximum of about 10.5% of the applied dose. In rabbits, 36% of a dose of 4 μg/cm^2 of 2,4-D in acetone applied to the shaved back was recovered in the urine over a 14-day period (Moody et al. 1990). In the same study with monkeys and rabbits, 15 and 29% of the dose applied to the forearm and forehead, respectively, was recovered in the urine over the same time period. Urinary excretion half-lives ranged from 1.47 days for the monkeys forehead application to 2.14 days for the rabbits back application.

In rats, fecal excretion of ^{14}C-2,4-D represented only a minor elimination route following dermal application of the chemical, with only 2% of the applied dose accounted for in the feces over a 14-day sampling period (Moody et al. 1994). In the same time period, guinea pigs excreted 9% of a dermal dose of 2,4-D in the feces (Moody et al. 1994). Mice applied a dose of 1 mg/kg ^{14}C-2,4-D in acetone on the shaved back excreted small amounts of radiolabel in the feces and as CO_2, although the authors did not provide the specific amounts (Grissom et al. 1985). In 24 hours, 93% of 2,4-D that had penetrated the application site (almost 21% of the applied dose) was accounted for in the excreta.

3.4.5 Physiologically Based Pharmacokinetic (PBPK)/Pharmacodynamic (PD) Models

Physiologically based pharmacokinetic (PBPK) models use mathematical descriptions of the uptake and disposition of chemical substances to quantitatively describe the relationships among critical biological processes (Krishnan et al. 1994). PBPK models are also called biologically based tissue dosimetry models. PBPK models are increasingly used in risk assessments, primarily to predict the concentration of potentially toxic moieties of a chemical that will be delivered to any given target tissue following various combinations of route, dose level, and test species (Clewell and Andersen 1985). Physiologically based pharmacodynamic (PBPD) models use mathematical descriptions of the dose-response function to quantitatively describe the relationship between target tissue dose and toxic end points.

PBPK/PD models refine our understanding of complex quantitative dose behaviors by helping to delineate and characterize the relationships between: (1) the external/exposure concentration and target tissue dose of the toxic moiety, and (2) the target tissue dose and observed responses (Andersen and Krishnan 1994; Andersen et al. 1987). These models are biologically and mechanistically based and can be used to extrapolate the pharmacokinetic behavior of chemical substances from high to low dose, from route to route, between species, and between subpopulations within a species. The biological basis of PBPK models results in more meaningful extrapolations than those generated with the more conventional use of uncertainty factors.

The PBPK model for a chemical substance is developed in four interconnected steps: (1) model representation, (2) model parameterization, (3) model simulation, and (4) model validation (Krishnan and Andersen 1994). In the early 1990s, validated PBPK models were developed for a number of toxicologically important chemical substances, both volatile and nonvolatile (Krishnan and Andersen 1994; Leung 1993). PBPK models for a particular substance require estimates of the chemical substance-specific physicochemical parameters, and species-specific physiological and biological parameters. The numerical estimates of these model parameters are incorporated within a set of differential and algebraic equations that describe the pharmacokinetic processes. Solving these differential and algebraic equations provides the predictions of tissue dose. Computers then provide process simulations based on these solutions.

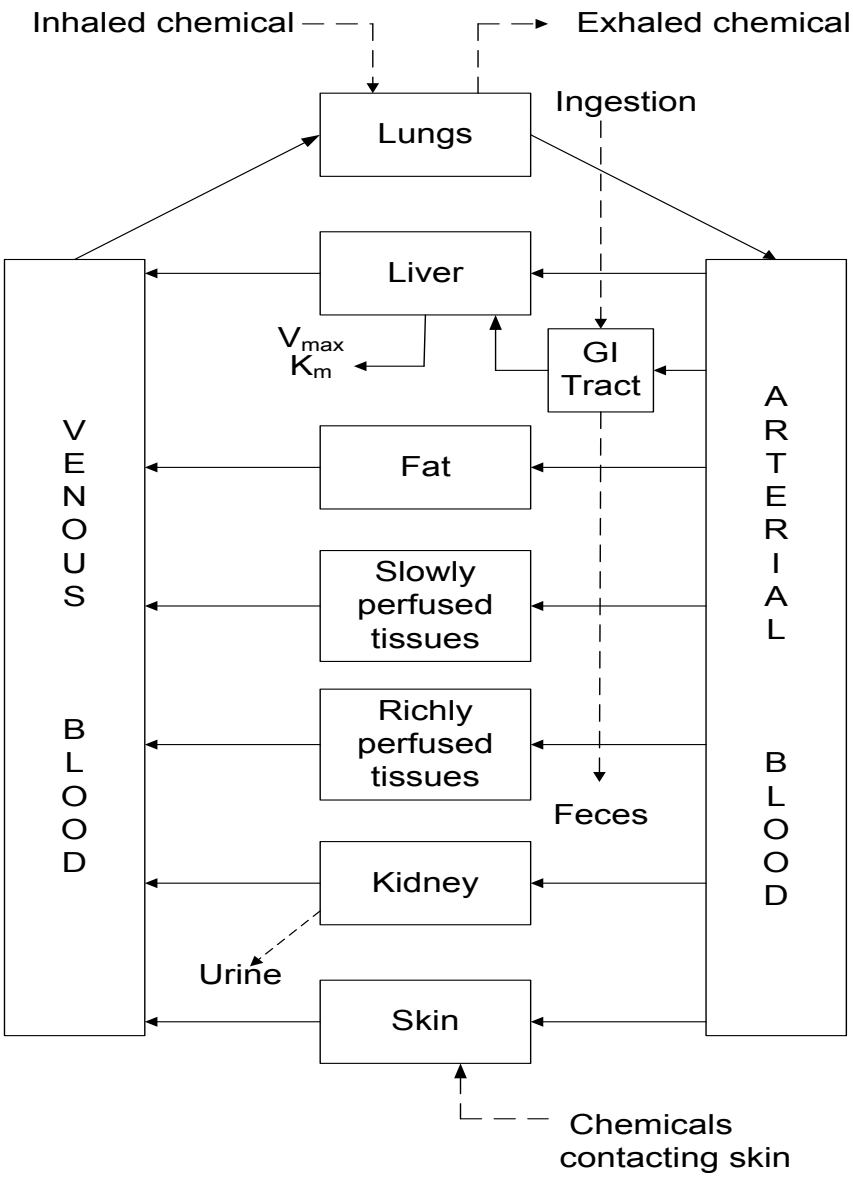
The structure and mathematical expressions used in PBPK models significantly simplify the true complexities of biological systems. However, if the uptake and disposition of the chemical substance(s) are adequately described, this simplification is desirable because data are often unavailable for many biological processes. A simplified scheme reduces the magnitude of cumulative uncertainty. The adequacy of the model is, therefore, of great importance, and model validation is essential to the use of PBPK models in risk assessment.

PBPK models improve the pharmacokinetic extrapolations used in risk assessments that identify the maximal (i.e., the safe) levels for human exposure to chemical substances (Andersen and Krishnan 1994). PBPK models provide a scientifically sound means to predict the target tissue dose of chemicals in humans who are exposed to environmental levels (for example, levels that might occur at hazardous waste sites) based on the results of studies where doses were higher or were administered in different species. Figure 3-4 shows a conceptualized representation of a PBPK model.

If PBPK models for 2,4-D exist, the overall results and individual models are discussed in this section in terms of their use in risk assessment, tissue dosimetry, and dose, route, and species extrapolations.

PBPK models for 2,4-D in rabbits, rats, and humans have been reported (Durkin et al. 2004; Kim et al. 1994, 1995, 1996, 2001). The Kim et al. (1994, 1995, 1996, 2001) models were developed with the primary objective of simulating regional brain distribution of 2,4-D. These models included compartments for various brain regions, while all other tissues were aggregated into a single compartment. The rat and human models developed by Durkin et al. (2004) have compartments for liver

Figure 3-4. Conceptual Representation of a Physiologically Based Pharmacokinetic (PBPK) Model for a Hypothetical Chemical Substance



Note: This is a conceptual representation of a physiologically based pharmacokinetic (PBPK) model for a hypothetical chemical substance. The chemical substance is shown to be absorbed via the skin, by inhalation, or by ingestion, metabolized in the liver, and excreted in the urine or by exhalation.

Source: Krishnan and Andersen 1994

and kidney, but no separate compartment for brain. The model has been applied to interspecies and route-to-route dosimetry calculations for deriving Hazard Quotients (ratio of a measure of exposure to a chemical to an establish benchmark such as a reference dose [RfD] or reference concentration [RfC]) in forestry workers who spray 2,4-D based on dose equivalence for plasma peak and average 2,4-D concentrations. The Durkin et al. (2004) and Kim et al. (2001) models differ in several other important ways. In the Durkin et al. (2004) model, exchanges of 2,4-D between plasma and tissues are flow-limited with partitioning of the non-ionized species (e.g., protonated acid) between interstitial and intracellular fluid in tissues. Uptake of the anionic base is attributed to differences in extracellular and intracellular pH which result in intracellular pH-trapping of the anionic base. In the Kim et al. (2001) models, exchanges between plasma and tissues are diffusion limited and no distinction is made between the protonated and anionic species. Another important difference concerns the simulation of urinary excretion of 2,4-D. In the Durkin et al. (2004) model, renal clearance of 2,4-D is dependent on plasma 2,4-D concentration with renal clearance decreasing as plasma 2,4-D concentration increases. This approach accommodated results of studies in animals that found dose-dependent inhibition of urinary excretion of 2,4-D. In the Kim et al. (2001) model, urinary excretion is simulated as capacity limited transfer of 2,4-D to urine. Both models include a "deep" compartment, which exchanges 2,4-D with plasma very slowly. In the Durkin et al. (2004) model, the deep compartment is assigned to red blood cells; in the Kim et al. (2001) models, the deep compartment is assigned a subcompartment of the lumped body compartment representing all tissues other than brain. The Durkin et al. (2004) model also includes parameters for simulating binding of 2,4-D to plasma protein. Although very different in structure, both models yielded similar predictions of plasma elimination kinetics when optimized to the same intravenous dosing studies in rats (Smith et al. 1980).

3.4.5.1 Discussion of Models

Rabbit (Kim et al. 2001)

Kim et al. (1994, 1995, 1996, 2001) developed a PBPK model for predicting uptake and distribution of 2,4-D in rabbit and rat brain. The model includes compartments for plasma, brain, and a single lumped body compartment representing all tissues other than brain. The brain compartment includes subcompartments representing the hypothalamus, caudate nucleus, hippocampus, forebrain, brainstem, cerebellum, brain plasma, and cerebrospinal fluid (CSF). The six brain compartments have distinct mass transfer clearance coefficients for plasma-brain and brain-CSF. The body compartment includes a deep subcompartment and a compartment representing the rest of the body (excluding brain). Exchanges of

2,4-D between plasma and brain are simulated as four process: (1) flow-limited exchange between central plasma and brain plasma, governed by blood flow and the brain/plasma partition coefficient; (2) diffusion-limited exchange between plasma and brain tissue governed by a mass transfer clearance coefficient; (3) diffusion-limited exchange between brain tissue and CSF; and (4) capacity-limited transfer from CSF to plasma, representing transport through the choroid plexus, governed by a V_{max} and K_m. Exchange of 2,4-D between plasma and the rest of the body is flow-limited. Excretion of 2,4,-D is represented as capacity-limited transfer from the body compartment (V_{max}, K_m).

Partition coefficients for the rabbit model were estimated from tissue/plasma concentration ratios measured in rabbits following a single intraperitoneal dose of 40 or 100 mg/kg ^{14}C-2,4-D (Kim et al. 1995). These same values were used in the rat model. Transfer coefficients for the rabbit model were optimized with data from the same study (Kim et al. 1995). Transfer coefficients for the rat model were optimized with data on plasma and brain concentrations in rats following intravenous injection of 10, 50, or 150 mg/kg 2,4-D or subcutaneous implantation of osmotic mini-pumps that delivered 2,4-D doses of 1 or 10 mg/kg day (Patterson et al. 2000; Smith et al. 1980). The rabbit model was evaluated by comparing observed and predicted time courses for plasma, CSF, and brain region 2,4-D concentrations. Data for an individual rabbit is displayed in Kim et al. (1995), and these plots show time profiles that are similar to observations. The rat model predicted plasma and brain regions concentration of 2,4-D that were within ±2 standard deviations of the mean observations (Kim et al. 2001).

A maternal-fetal model was developed based on the rabbit model (Kim et al. 1996). The model includes placental and amniotic fluid compartments and fetal tissue compartments representing fetal CSF, fetal brain tissue, and fetal brain plasma. Exchanges between maternal plasma and placenta are flow-limited. Exchanges between fetal plasma and brain include the same four flow-limited, diffusion-limited, and capacity-limited processes as in the maternal model. 2,4-D in amniotic fluid undergoes diffusion-limited exchange with 2,4-D in the fetal body compartment and with the placenta. Transfer coefficients were optimized based on data from a study in which anesthetized pregnant rabbits received intravenous doses of 1, 10, or 40 mg/kg ^{14}C-2,4-D on GD 30. The study provided time-course data on 2,4-D in maternal and fetal plasma, amniotic fluid, and fetal brain. The optimized model predicted the dose-dependent time course for 2,4-D fetal and maternal plasma, amniotic fluid, fetal brain, and maternal brain regions.

Human and Rat (Durkin et al. 2004)

Durkin et al. (2004) developed a PBPK model of 2,4-D for predicting internal exposures resulting from ingestion exposures in rats and dermal exposures in humans. The model includes compartments for plasma, red blood cells, skin, kidney, liver, gastrointestinal tract, and a lumped compartment representing other tissues. The blood compartment includes a red cell compartment which exchanges 2,4-D slowly with plasma (first order). The plasma compartment includes saturable binding to two classes of binding sites. The free unbound fraction exchanges with tissues. Exchanges of 2,4-D between plasma and tissues are flow-limited with partitioning of the non-ionized species (e.g., protonated acid) between interstitial and intracellular fluid in tissues. Dissociation of the acid into its anionic base is calculated based on the Henderson-Hasselbalch equation, pKa for 2,4-D (2.87) and pH of interstitial fluid (7.0) and intracellular fluid (7.4). The lower intracellular pH favors intracellular trapping of the anion. The liver compartment includes a term for first-order transfer of 2,4-D into the gastrointestinal tract representing biliary secretion. Excretion of 2,4-D is simulated as four processes: (1) delivery of 2,4-D into tubule fluid from glomerular filtration; (2) saturable transport of the anionic base from plasma into kidney (V_{max}, K_m); (3) secretion of the anionic based from kidney into urine (first order); and (4) excretion of 2,4-D in tubule fluid into urine (first order). Studies conducted in animals have found that urinary excretion of 2,4-D is inhibited by increasing concentrations of plasma 2,4-D (Orberg 1980; Smith et al. 1980, unpublished). Although the mechanism for this apparent self-inhibition in not understood, the inhibition affects both glomerular filtration and renal secretion of 2,4-D, suggesting that it may represent a vascular effect resulting in depression of glomerular filtration and/or renal blood flow (Durkin et al. 2004). The pharmacodynamics of inhibition of urinary excretion are represented in the model as an adjustment to parameters that govern glomerular filtration, transport from plasma into kidney, and secretion of 2,4-D into urine. The adjustment factor is a variable that changes in value as a function of plasma 2,4-D concentration. Dependence of the adjustment factor on plasma 2,4-D concentration results in renal clearance of 2,4-D decreasing with increasing plasma 2,4-D concentration. The adjustment factor was empirically derived from animal studies (Orberg 1980). Absorption pathways in the model are from the gastrointestinal tract and skin surface. The gastrointestinal tract model incudes compartments representing stomach lumen, gastrointestinal tract lumen (representing the tract distal to stomach), and gastrointestinal tract tissue. Absorption from the stomach and transfer to feces are first-order processes. 2,4-D deposited on skin is subject to first-order transfer to the environment (fugitive loss) or first-order absorption into skin tissue from where it can undergo flow limited exchange with plasma.

The model was parameterized to simulate rats, and subsequently extrapolated to humans. The rat model was based primarily on intravenous and oral studies (Smith et al. 1980, unpublished). Rats were administered a single intravenous dose (5 or 90 mg/kg) or oral dose (10, 25, 50, or 150 mg/kg). A study conducted in goats was used to estimate the effects of 2,4-D dose on 2,4-D renal clearance and glomerular filtration (Orberg 1980). Protein binding parameters were based on data from studies conducted in rats (Ylatalo et al. 1990), goats (Orberg 1980), and bovine serum albumin (Kolberg et al. 1973). Partition coefficients were estimated from physical-chemical properties of 2,4-D and tissue composition (Poulin and Krishnan 1995) and adjusted based on measured values for brain/plasma (Kim et al. 1995). The rat model was initially optimized based on data from the rat intravenous study and then applied to the rat oral study to estimate values for gastrointestinal tract absorption parameters. By parameterizing the model to achieve decreasing renal clearance in association with increasing plasma 2,4-D concentrations, the model predicted the observed nonlinear dose-dependence of urinary excretion and plasma concentration as well as time-dependent changes in kinetics of 2,4-D removal from plasma and excretion in urine following dosing (Smith et al. 1980).

The human model was optimized to data from studies conducted in humans (Feldmann and Maibach, 1974; Sauerhoff et al. 1977). In the Feldmann and Maibach (1977) study, urinary ^{14}C was measured following a single intravenous (tracer) dose of ^{14}C-2,4-D or dermal dose to the forearm (4 µg/cm^2). In the Sauerhoff et al. (1977) study, plasma levels and urinary excretion of 2,4-D were measured following a single oral dose of 2,4-D (5 mg/kg). Data from the human studies were used to optimize values for parameters controlling the absorption rate from the gastrointestinal tract, absorption rate from skin, V_{max} uptake to kidney, and k_e for urinary excretion. All other parameters were allometrically scaled from the rat model.

The human model was evaluated by comparing observed and predicted urinary excretion of 2,4-D in forestry workers who sprayed 2,4-D from backpack sprayers (Lavy et al. 1984, 1987). The study provided data on application rates and urinary excretion of 2,4-D over a 5-day period. Skin deposition rates were estimated from data contained in the Pesticide Handlers Exposure Database (PHED Task Force 1995). Predictions from the optimized model encompassed observed cumulative urinary excretion of 2,4-D.

The model was applied to an interspecies and route-to-route dosimetry extrapolation. The model was used to predict plasma 2,4-D concentrations corresponding to a rat NOAEL and LOAEL estimated from a 90-day feeding study (Serota et al. 1983). Average and peak plasma concentrations in rats corresponding

to the NOAEL were predicted to be 3.6 and 7.2 µM, respectively. Average (2-week) plasma 2,4-D concentrations in forestry workers were predicted to range from 1.4 to 7.3 µM and peak concentration were predicted to range from 2.5 to 13 µM.

3.5 MECHANISMS OF ACTION

3.5.1 Pharmacokinetic Mechanisms

Absorption. No information was located regarding specific mechanisms of absorption of 2,4-D through the gastrointestinal tract or the skin. Because 2,4-D and the simple salts exist predominantly in the ionized form at physiological pH, it does not readily move across the lipid bilayer of the cellular membranes. Therefore, active transport mechanisms of the parent anion must be involved in its entry into cells. Active transport translocation of 2,4-D has been demonstrated, for example, in studies with the choroid plexus from rabbits (Kim and O'Tuama 1981; Kim et al. 1983; Pritchard 1980), with renal cortical tissue from rats and rabbits (Berndt and Koschier 1973), and Chinese hamster ovary cells (Bergesse and Balegno 1995).

Distribution. Studies in animals have shown that once absorbed, 2,4-D is transported highly bound to proteins in plasma, particularly albumin, which is subject to saturable protein binding with large exposures. Although protein binding has not been directly shown in humans, Fang and Lindstrom (1980) reported that 2,4-D could bind *in vitro* to serum albumin from eight different mammalian species, including human serum albumin. The binding affinities varied among species. Affinity seemed to be the highest for human albumin followed by rat, horse, ovine, porcine, chicken, and guinea pig. Others have also reported binding of 2,4-D to bovine serum albumin (Haque et al. 1975; Kolberg et al. 1973) and to human serum albumin *in vitro* (Rosso et al. 1998). The latter investigators noted that the binding affinity of 2,4-D to human serum albumin was several times higher than the affinity found for common pharmaceutical compounds. An *in vitro* study showed that incubation of rat plasma with 0.5 mg 2,4-D resulted in 28.3% of the 2,4-D unbound to protein, which increased to 42% as the concentrations of 2,4-D in the medium was increased to 1.0 mg, suggesting saturation of the binding process under the conditions of the study (Tyynelä et al. 1990). In an *in vivo* study in male and female rats, determination of plasma protein binding at concentrations of 2,4-D of 6, 24, and 48 µg/mL showed that approximately 97% of the chemical was bound in both sexes (Griffin et al. 1977a). Another study reported that plasma protein binding values for rats dosed 5 or 50 mg/kg 2,4-D were 95.5 and 92.9%, respectively (van Ravenszwaay et al. 2003). The respectively values for dogs were 95.7 and 87.6%.

Metabolism. As indicated in Section 3.4.3, Metabolism, 2,4-D undergoes limited metabolism in humans and animals. There is no evidence that the limited metabolism of 2,4-D leads to the formation of toxic metabolites.

Excretion. 2,4-D is eliminated from the body mainly by excretion in the urine. Because of extensive protein binding in plasma over a wide range of concentrations (Griffin et al. 1997a; van Ravenszwaay et al. 2003), protein-bound 2,4-D is not readily filtered at the glomerulus, but it is actively secreted into urine by means of an OAT1 carrier protein located on the basolateral membrane of the renal proximal tubules. The carrier is saturable and the point of saturation varies between animal species, sex within species, and life-stage. Studies have shown that in rats, saturation occurs following single oral doses in excess of 50 mg/kg 2,4-D (Gorzinski et al. 1987; Saghir et al. 2013). Adult male rats express higher levels of OAT1 than adult females (Buist et al. 2002), which is consistent with an increased susceptibility of female rats to 2,4-D-induced renal lesions than male rats (Marty et al. 2013). The latter investigators suggested that saturation of the OAT1 at lower 2,4-D plasma concentrations than in males would preferentially decrease the delivered dose of 2,4-D to the proximal tubules in females relative to males. The differential expression of OAT1 in males and female rats is also consistent with females showing a significantly lower rate of elimination from plasma, lower volume of distribution, and higher elimination half-life than males (Griffin et al. 1997a; see also Section 3.4.2.2 for higher distribution to tissues in female rats compared with male rats). The OAT1 carrier was also found to be developmentally-regulated, as expression increased 4-fold between PNDs 5 and 35 in both males and female rats (Buist et al. 2002). However, expression of more OAT1 messenger RNA in males than in females by PND 40 (Buist et al. 2002) could explain the findings of Saghir et al. (2013) of lower renal clearance in females than in male pups on PND 35.

Comparative studies have shown that dogs have a slower renal clearance for 2,4-D and other organic acids than other species, including humans (Timchalk 2004). Following oral doses of 1–5 mg 2,4-D/kg, plasma half-life in dogs ranged from 31 to 92–106 hours. In contrast, plasma half-lives ranged from 0.8 to 12 hours in mice, rats, pigs, calves, and humans. Comparative analyses using allometric equations to scale between species based on body weight showed that volume of distribution, renal clearance, and elimination half-life increased linearly with body weight in all species tested except dogs. Renal clearance in dogs was slower than in other species and was not adequately described by scaling. Elimination half-life in dogs also was higher than in other species and was not well described by scaling. Timchalk (2004) proposed that the sensitivity of the dog to the toxicity of 2,4-D is primarily due to the

dog's relatively low capacity to excrete organic acids and suggested that dogs might not be a relevant species for evaluation of human health risk.

3.5.2 Mechanisms of Toxicity

The role of oxidative stress in the toxicity of 2,4-D has been explored in a few studies. Twenty-five-day-old offspring from rats exposed to 100 mg 2,4-D/kg/day from PND 9 to 25 showed significantly increases in reactive oxygen species in the midbrain, striatum, and prefrontal cortex (Ferri et al. 2007). Less marked effects were reported in the hippocampus and no effects were noted in the hypothalamus. The different sensitivities between tissues was attributed by the investigators to different enzyme activities profiles, different levels of copper or iron ions, which are involved in oxidative stress generation, and/or the high flux of reactive oxygen species generated during neurochemical reactions. Indicators of oxidative stress were increased and antioxidant enzyme levels were reduced in the liver from rats and their pups following maternal exposure to 126 mg 2,4-D/kg/day from GD 14 to PND 14 (Troudi et al. 2012a). Increased oxidative stress, decreased antioxidant enzyme activity, and decreased levels of non-enzymatic antioxidant levels were seen in hemolysate and bone homogenates from offspring from rats dosed in the same manner (Troudi et al. 2012b). In yet another study, exposure of rats to 100 mg 2,4-D/kg/day on GDs 1–19 resulted in increased levels of malondialdehyde and reduced levels of antioxidant enzymes in the liver of dams and fetuses on GD 20; this was partially prevented by treatment of the dams with vitamin E (Mazhar et al. 2014). Treatment of mice with 2,4-D in drinking water in doses of up to 100 mg 2,4-D/kg/day on GDs 0–9 did not induce signs of oxidative stress in maternal blood collected on GD 9 (Dinamarca et al. 2007).

Bradberry et al. (2000) reviewed the toxicity of chlorophenoxy herbicides and suggested three modes of action that could be potentially involved, namely, effects associated with the plasma membrane, interference in cellular metabolic pathways involving acetylcoenzyme A (AcCoA), and uncoupling of oxidative phosphorylation as a result of disruption of cellular membranes by the herbicide. The summary below is taken from Bradberry's review; the reader is referred to references cited therein for more detailed information.

Support for alterations to plasma membranes comes from studies showing chlorophenoxy herbicide-induced alterations to model membrane systems, *in vitro* human erythrocyte cell membranes, disruption of cell membrane transport mechanisms, and inhibition of ion channels. Because chlorophenoxyacetic acids are able to form analogues of AcCoA *in vitro*, the potential exists for such analogues to disrupt

cellular metabolic pathways involving AcCoA, such as the synthesis of the neurotransmitter acetylcholine. The formation of a choline ester that could act as a false transmitter would affect muscarinic and nicotinic synapses. Similarly affected could be other metabolic pathways of AcCoA resulting in interference with energy metabolism and the citric acid cycle. Studies *in vitro* have shown that phenoxy herbicides can uncouple oxidative phosphorylation, thus compromising a variety of cellular activities, including the ability of the cell to maintain ionic gradients across membranes, DNA and protein synthesis, and polymerization of microtubules and microfilaments leading to disruption of the cytoskeleton and altering cell shape. Some effects reported in humans following poisoning with phenoxy herbicide formulations and in animals following exposure to high doses of 2,4-D, such as damage to the blood-brain barrier, myotonia, and muscle twitching, are consistent with modes of actions described above.

A series of studies have been conducted by Evangelista de Duffard and coworkers examining neurochemical alterations in the brain from both adult rats and from offspring of dams exposed to 2,4-D during gestation and lactation. In some of these studies, rats were treated orally and in other studies, rats were dosed by intraperitoneal injection. Doses tested were ≥50 mg 2,4-D/kg/day. A brief summary of the findings follows.

Exposure to 2,4-D induced behavioral alterations in adult rats through serotonergic and dopaminergic mechanisms and interacted with amphetamine to induce a 'Serotonergic Syndrome' (a behavioral response induced in rodents by stimulation of serotonergic receptors) and additional dopaminergic stimulation; female rats appeared to be more affected than males (Evangelista de Duffard et al. 1995). The behavioral alterations in the presence of amphetamine appeared to be due to increased content of serotonin and dopamine in the substantia nigra, ventral tegmental area, nucleus accumbens, striatum, midbrain, and cerebellum (Bortolozzi et al. 1998). The investigators hypothesized that the increase in serotonin and dopamine in amphetamine-challenged rats could occur because the neurons remain hyperactive after 2,4-D treatment and amphetamine initiates an immediate release of serotonin and dopamine to the extracellular fluid (Bortolozzi et al. 1998).

In another study, the investigators showed that rat offspring exposed to 2,4-D through the placenta and the dams' milk followed by direct exposure showed neurobehavioral alterations that seemed to disappear as adults (Bortolozzi et al. 1999). In offspring exposed during gestation and lactation, 2,4-D also induced neurobehavioral alterations, some of which could be unmasked with pharmacological challenges (Bortolozzi et al. 1999). Dopamine D_2 receptors appeared to be implicated in the stimulant-induced

behavioral sensitization (Bortolozzi et al. 2002). Further studies showed that in 2,4-D-exposed rats, dopamine D2 receptors were increased in density by about 40% in the striatum of rats exposed perinatally and then directly, but were also increased in the prefrontal cortex and cerebellum; females appeared more affected than males (Bortolozzi et al. 2004).

Studies also showed that exposure to 2,4-D *in utero* and through lactation produced a permanent increase in serotonergic neurons in all mesencephalic nuclei from offspring (Garcia et al. 2001). However, perinatal exposure followed by direct exposure resulted in only an increase in serotonergic neurons from the dorsal raphe nuclei, suggesting an adaptable response of serotonergic neurons in the median raphe nucleus. In addition, the immunocytochemically-detected glial reaction was different for the two exposure designs. Further studies showed that levels of dopamine and dopamine metabolites were decreased in the right side with respect to the left side in the striatum and nucleus accumbens in rats exposed perinatally and then directly, which seemed to provide support for the rotation motion exhibited by these rats (Bortolozzi et al. 2003). In subsequent studies of rat pups exposed via lactation, the investigators suggested that 2,4-D decreased tyrosine hydroxylase (enzyme that catalyzes the rate limiting step in this synthesis of catecholamines) immunoreactivity in the substantia nigra and ventral segmental area in the midbrain resulting in a significant diminution in serotonin fiber density (Garcia et al. 2004, 2006).

Injection of 2,4-D into various brain areas of adult rats showed different behavioral alterations possibly by exerting different types of interactions with the monoaminergic system depending on the location of the 2,4-D injection and dose and time period post-injection. Toxicity of 2,4-D appeared to differ between monoaminergic terminals, axonal fibers, and cell bodies (Bortolozzi et al. 2001).

Other studies from the same group of investigators showed that behavioral alterations could be related to induction of reactive gliosis in the hippocampus and cerebellum from rat pups exposed through maternal milk (Brusco et al. 1997), altering myelin deposition and ganglioside pattern in various brain areas from rat pups treated directly with 2,4-D (Rosso et al. 1997, 2000a) or through maternal milk (Duffard et al. 1996). They also showed that 2,4-D can disrupt microtubule assembly and disorganize the Golgi apparatus in cultured cerebellar granule cells *in vitro* possibly leading to decreased neurite outgrowth (Rosso et al. 2000b).

3.5.3 Animal-to-Human Extrapolations

As mentioned previously, it has been proposed that the dog might not be a relevant species for evaluation of human health risk because of the relatively low capacity to excrete 2,4-D (Timchalk 2004). The implication is that, at equivalent doses of 2,4-D, more 2,4-D will remain in plasma and potentially reach tissues in dogs than in other species, particularly at lower doses since clearance may become saturated in most species at higher doses. This was illustrated by van Ravenzwaay et al. (2003) who reported that equivalent doses of 5 and 50 mg 2,4-D/kg given to rats and dogs resulted in plasma 2,4-D AUCs 125- and 15-fold greater, respectively, in dogs than in rats.

3.6 TOXICITIES MEDIATED THROUGH THE NEUROENDOCRINE AXIS

Recently, attention has focused on the potential hazardous effects of certain chemicals on the endocrine system because of the ability of these chemicals to mimic or block endogenous hormones. Chemicals with this type of activity are most commonly referred to as *endocrine disruptors*. However, appropriate terminology to describe such effects remains controversial. The terminology *endocrine disruptors*, initially used by Thomas and Colborn (1992), was also used in 1996 when Congress mandated the EPA to develop a screening program for "...certain substances [which] may have an effect produced by a naturally occurring estrogen, or other such endocrine effect[s]...". To meet this mandate, EPA convened a panel called the Endocrine Disruptors Screening and Testing Advisory Committee (EDSTAC), and in 1998, the EDSTAC completed its deliberations and made recommendations to EPA concerning *endocrine disruptors*. In 1999, the National Academy of Sciences released a report that referred to these same types of chemicals as *hormonally active agents*. The terminology *endocrine modulators* has also been used to convey the fact that effects caused by such chemicals may not necessarily be adverse. Many scientists agree that chemicals with the ability to disrupt or modulate the endocrine system are a potential threat to the health of humans, aquatic animals, and wildlife. However, others think that endocrine-active chemicals do not pose a significant health risk, particularly in view of the fact that hormone mimics exist in the natural environment. Examples of natural hormone mimics are the isoflavinoid phytoestrogens (Adlercreutz 1995; Livingston 1978; Mayr et al. 1992). These chemicals are derived from plants and are similar in structure and action to endogenous estrogen. Although the public health significance and descriptive terminology of substances capable of affecting the endocrine system remains controversial, scientists agree that these chemicals may affect the synthesis, secretion, transport, binding, action, or elimination of natural hormones in the body responsible for maintaining homeostasis, reproduction, development, and/or behavior (EPA 1997). Stated differently, such compounds may cause toxicities that are mediated through the neuroendocrine axis. As a result, these chemicals may play a role in altering,

for example, metabolic, sexual, immune, and neurobehavioral function. Such chemicals are also thought to be involved in inducing breast, testicular, and prostate cancers, as well as endometriosis (Berger 1994; Giwercman et al. 1993; Hoel et al. 1992).

There are no studies in humans that would suggest that 2,4-D is an endocrine disruptor chemical. Studies in animals or *in vitro* assays suggest that 2,4-D is not an endocrine disruptor. Although altered behavioral effects may occur (Bortolozzi et al. 2001; Duffard et al. 1996; Rosso et al. 2000a, 2000b) that indicate a disruption, it is unlikely that they occurred through endocrine pathways.

Studies in animals summarized in Section 3.2.2, Oral Exposure, did not find morphological alterations in endocrine glands following exposure to 2,4-D. One study reported a significant reduction in serum prolactin levels in rats dosed with ≥15 mg 2,4-D/kg/day via the drinking water on postpartum days 1–7 (Stürtz et al. 2008). The investigators suggested that alterations in levels of serotonin and dopamine in the arcuate nucleus of the brain may have been responsible for the reduction in serum prolactin. Some studies reported decreased serum levels of T4 in rats exposed to relatively high doses of 2,4-D (i.e., Charles et al. 1996a; Gorzinski et al. 1987), which could have been due to competition of 2,4-D with T4 for binding with plasma proteins. None of these studies reported histopathological changes in the thyroid gland. In an F1-extended 1-generation reproductive study in rats, there was no evidence that 2,4-D had androgenic, anti-androgenic, estrogenic, or anti-estrogenic activity (Marty et al. 2013).

Badawi et al. (2000) reported that gavage administration of a single dose of 375 mg 2,4-D/kg to rats induced the expression of cytochromes CYP1A1, CYP1A2, and CYP1B1, which resulted in increased metabolism of estrogen in liver, kidney, and mammary gland. It should be noted, however, that 375 mg/kg is a high dose of 2,4-D, at least half of the oral LD_{50} dose for rats, and is unlikely to be encountered in environmental exposures to 2,4-D.

2,4-D did not bind to the androgen receptor (AR) in an *in vitro* AR bindings assay using a recombinant rat AR (Fang et al. 2003). 2,4-D showed no estrogenic or anti-estrogenic activity in a two hybrid assay system or anti-estrogenic activity in a reporter gene assay system using MCF-7 cells (Jung et al. 2004; Nishihara et al. 2000). 2,4-D did not show estrogenicity in other studies using MCF-7 breast cancer cells (Lin and Garry 2000; Soto et al. 1995). 2,4-D did not show estrogenic activity in a competitive-binding assay utilizing estrogen receptor from uteri from ovariectomized rats (Blair et al. 2000). Orton et al. (2009) reported that 2,4-D did not exhibit estrogenic, androgenic, anti-estrogenic, or anti-androgenic activity in a recombinant yeast assay *in vitro* at environmentally relevant concentrations. Similar negative

results were shown in an *in vitro* reporter gene assay using Chinese hamster ovary cells (Kojima et al. 2004). 2,4-D did not show androgenic activity in human prostate cancer cells *in vitro* and had no significant effect on either mRNA or protein levels of AR; however, 2,4-D with 5α-dihydroxytestosterone showed synergistic androgenic activity through, in part, the promotion of AR nuclear translocation (Kim et al. 2005).

The EPA recently completed a weight-of-evidence analysis of the potential interaction of 2,4-D with the androgen, estrogen, and thyroid signaling pathways and concluded that there is no convincing evidence of interaction with either of the three pathways (EPA 2015c, 2015d). Specifically, results from an *in vitro* AR binding assay using rat prostate cytosol showed that 2,4-D was negative for AR binding at concentrations up to 10^{-4} M. In an *in vitro* aromatase assay, aromatase activity for 2,4-D was similar to full activity controls at all concentrations of 2,4-D tested. The results from an ER binding assay using rat uterine cytosol showed that 2,4-D was negative for ER binding at concentrations of up to 10^{-4} M. Results from an *in vitro* estrogen receptor transcriptional activation assay in a human cell line indicated that 2,4-D treatment did not result in ER-mediated transcriptional activation at any concentration relevant for use in the assay. In an *in vitro* steroidogenesis assay using human adrenocortical carcinoma cells, 2,4-D treatment produced a statistically significant increase in estradiol production at the assay limit-concentration of 10^{-4} M. Because the increase in estradiol production did not meet the 1.5-fold cut off established in the validation program for the assay, it was not considered biologically relevant. 2,4-D did not significantly affect testosterone production at any concentration tested.

3.7 CHILDREN'S SUSCEPTIBILITY

This section discusses potential health effects from exposures during the period from conception to maturity at 18 years of age in humans, when most biological systems will have fully developed. Potential effects on offspring resulting from exposures of parental germ cells are considered, as well as any indirect effects on the fetus and neonate resulting from maternal exposure during gestation and lactation. Relevant animal and *in vitro* models are also discussed.

Children are not small adults. They differ from adults in their exposures and may differ in their susceptibility to hazardous chemicals. Children's unique physiology and behavior can influence the extent of their exposure. Exposures of children are discussed in Section 6.6, Exposures of Children.

Children sometimes differ from adults in their susceptibility to adverse health effects from exposure to hazardous chemicals, but whether there is a difference depends on the chemical(s) (Guzelian et al. 1992; NRC 1993). Children may be more or less susceptible than adults to exposure-related health effects, and the relationship may change with developmental age (Guzelian et al. 1992; NRC 1993). Vulnerability often depends on developmental stage. There are critical periods of structural and functional development during both prenatal and postnatal life that are most sensitive to disruption from exposure to hazardous substances. Damage from exposure in one stage may not be evident until a later stage of development. There are often differences in pharmacokinetics and metabolism between children and adults. For example, absorption may be different in neonates because of the immaturity of their gastrointestinal tract and their larger skin surface area in proportion to body weight (Morselli et al. 1980; NRC 1993); the gastrointestinal absorption of lead is greatest in infants and young children (Ziegler et al. 1978). Distribution of xenobiotics may be different; for example, infants have a larger proportion of their bodies as extracellular water, and their brains and livers are proportionately larger (Altman and Dittmer 1974; Fomon 1966; Fomon et al. 1982; Owen and Brozek 1966; Widdowson and Dickerson 1964). Past literature has often described the fetus/infant as having an immature (developing) blood-brain barrier that is leaky and poorly intact (Costa et al. 2004). However, current evidence suggests that the blood-brain barrier is anatomically and physically intact at this stage of development, and the restrictive intracellular junctions that exist at the blood-CNS interface are fully formed, intact, and functionally effective (Saunders et al. 2008, 2012).

However, during development of the brain, there are differences between fetuses/infants and adults that are toxicologically important. These differences mainly involve variations in physiological transport systems that form during development (Ek et al. 2012). These transport mechanisms (influx and efflux) play an important role in the movement of amino acids and other vital substances across the blood-brain barrier in the developing brain; these transport mechanisms are far more active in the developing brain than in the adult. Because many drugs or potential toxins may be transported into the brain using these same transport mechanisms—the developing brain may be rendered more vulnerable than the adult. Thus, concern regarding possible involvement of the blood-brain barrier with enhanced susceptibility of the developing brain to toxins is valid. It is important to note however, that this potential selective vulnerability of the developing brain is associated with essential normal physiological mechanisms; and not because of an absence or deficiency of anatomical/physical barrier mechanisms.

The presence of these unique transport systems in the developing brain of the fetus/infant is intriguing; whether these mechanisms provide protection for the developing brain or render it more vulnerable to

toxic injury is an important toxicological question. Chemical exposure should be assessed on a case-by-case basis. Research continues into the function and structure of the blood-brain barrier in early life (Kearns et al. 2003; Saunders et al. 2012; Scheuplein et al. 2002).

Many xenobiotic metabolizing enzymes have distinctive developmental patterns. At various stages of growth and development, levels of particular enzymes may be higher or lower than those of adults, and sometimes unique enzymes may exist at particular developmental stages (Komori et al. 1990; Leeder and Kearns 1997; NRC 1993; Vieira et al. 1996). Whether differences in xenobiotic metabolism make the child more or less susceptible also depends on whether the relevant enzymes are involved in activation of the parent compound to its toxic form or in detoxification. There may also be differences in excretion, particularly in newborns given their low glomerular filtration rate and not having developed efficient tubular secretion and resorption capacities (Altman and Dittmer 1974; NRC 1993; West et al. 1948). Children and adults may differ in their capacity to repair damage from chemical insults. Children also have a longer remaining lifetime in which to express damage from chemicals; this potential is particularly relevant to cancer.

Certain characteristics of the developing human may increase exposure or susceptibility, whereas others may decrease susceptibility to the same chemical. For example, although infants breathe more air per kilogram of body weight than adults breathe, this difference might be somewhat counterbalanced by their alveoli being less developed, which results in a disproportionately smaller surface area for alveolar absorption (NRC 1993).

Epidemiological studies of farming communities where 2,4-D has been used, which have included monitoring of children, have not provided convincing evidence of associations between 2,4-D and adverse health outcomes in children. For example, no significant association was found between 2,4-D and birth weight in the AHS (Sathyanarayana et al. 2010), birth defects in the Ontario Farm Family Health Study (Weselak et al. 2008), or birth defects and congenital anomalies in a study of pesticide applicators in the San Joaquin Valley of California (Yang et al. 2014). Studies of state-licensed, private pesticide applicators in Minnesota found a significant increase in birth defects among children conceived during the herbicide application season (Garry et al. 1996, 2002). However, chemical-specific analyses were not conducted.

Further evaluation of children born to participants in the Ontario Farm Family Health Study showed a significant increased risk of hay fever or allergies associated with maternal exposure to 2,4-D during

pregnancy among male offspring, but not among female offspring (Weselak et al. 2007). No significant association was found between exposure to 2,4-D and asthma or persistent cough or bronchitis.

Studies of children from parents participants in the AHS did not find significant associations between 2,4-D exposure and NHL, Hodgkin's disease, or leukemia (Flower et al. 2004). In a study of exposure to 2,4-D in house dust in California, childhood leukemia was not associated with 2,4-D (Metayer et al. 2013).

Animal studies have shown that 2,4-D can be transferred to the offspring through the placenta and via the mother's milk and that it distributes widely in fetal or neonatal tissues (Lindquist and Ullberg 1971; Marty et al. 2013; Saghir et al. 2013; Sandberg et al. 1996; Stürtz et al. 2000, 2006). Therefore, it seems reasonable to assume that the same could happen in humans.

As summarized in Section 3.2.2.6, Developmental Effects, studies in rodents have shown that, for the most part, adverse developmental effects (i.e., mainly reduced body weight in the offspring) occur at maternal dose levels that induced maternal toxicity, mainly reduced maternal weight during pregnancy. Reduced offspring weight was reported in a study in rats administered a relatively low postpartum dose of 2.5 mg 2,4-D/kg/day (Stürtz et al. 2010). This was attributed to 2,4-D affecting the suckling-induced hormone release milk transfer to the litter. However, no such effect has been reported in other studies that exposed dams to considerably higher doses (approximately 29 mg 2,4-D/kg/day) for periods that included gestation and postpartum (Marty et al. 2013).

2,4-D has not been found to cause teratogenicity in animal studies (Charles et al. 2001; Marty et al. 2013; Schwetz et al. 1971).

3.8 BIOMARKERS OF EXPOSURE AND EFFECT

Biomarkers are broadly defined as indicators signaling events in biologic systems or samples. They have been classified as markers of exposure, markers of effect, and markers of susceptibility (NAS/NRC 1989).

The National Report on Human Exposure to Environmental Chemicals provides an ongoing assessment of a generalizable sample of the exposure of the U.S. population to environmental chemicals using biomonitoring. This report is available at http://www.cdc.gov/exposurereport/. The biomonitoring data

for 2,4-D from this report is discussed in Section 6.5. A biomarker of exposure is a xenobiotic substance or its metabolite(s) or the product of an interaction between a xenobiotic agent and some target molecule(s) or cell(s) that is measured within a compartment of an organism (NAS/NRC 1989). The preferred biomarkers of exposure are generally the substance itself, substance-specific metabolites in readily obtainable body fluid(s), or excreta. However, several factors can confound the use and interpretation of biomarkers of exposure. The body burden of a substance may be the result of exposures from more than one source. The substance being measured may be a metabolite of another xenobiotic substance (e.g., high urinary levels of phenol can result from exposure to several different aromatic compounds). Depending on the properties of the substance (e.g., biologic half-life) and environmental conditions (e.g., duration and route of exposure), the substance and all of its metabolites may have left the body by the time samples can be taken. It may be difficult to identify individuals exposed to hazardous substances that are commonly found in body tissues and fluids (e.g., essential mineral nutrients such as copper, zinc, and selenium). Biomarkers of exposure to 2,4-D are discussed in Section 3.8.1.

Biomarkers of effect are defined as any measurable biochemical, physiologic, or other alteration within an organism that, depending on magnitude, can be recognized as an established or potential health impairment or disease (NAS/NRC 1989). This definition encompasses biochemical or cellular signals of tissue dysfunction (e.g., increased liver enzyme activity or pathologic changes in female genital epithelial cells), as well as physiologic signs of dysfunction such as increased blood pressure or decreased lung capacity. Note that these markers are not often substance specific. They also may not be directly adverse, but can indicate potential health impairment (e.g., DNA adducts). Biomarkers of effects caused by 2,4-D are discussed in Section 3.8.2.

A biomarker of susceptibility is an indicator of an inherent or acquired limitation of an organism's ability to respond to the challenge of exposure to a specific xenobiotic substance. It can be an intrinsic genetic or other characteristic or a preexisting disease that results in an increase in absorbed dose, a decrease in the biologically effective dose, or a target tissue response. If biomarkers of susceptibility exist, they are discussed in Section 3.10, Populations That Are Unusually Susceptible.

3.8.1 Biomarkers Used to Identify or Quantify Exposure to 2,4-D

As mentioned in Section 3.3, Metabolism, 2,4-D undergoes limited metabolism in humans and can thus be measured as unchanged parent compound in body fluids and tissues from humans. Information regarding levels in human tissues is available from cases of acute intentional or accidental oral

intoxication with commercial products that contained 2,4-D that resulted in deaths (i.e., Dudley and Thapar 1972; Keller et al. 1994; Nielsen et al. 1965; Osterloh et al. 1983). Tissue levels of 2,4-D determined in these and other case reports are typically not representative of occupational or environmental exposure to 2,4-D.

2,4-D can be readily measured in urine (see Chapter 7), and with the benefit of non-invasive collection procedure, urine is a widely used and accepted media to ascertain exposure to 2,4-D. Because 2,4-D is rapidly eliminated from the body (Kohli et al. 1974; Sauerhoff et al. 1977), urinary levels of 2,4-D reflect recent exposure, within days.

There are many reports that provide information regarding urinary levels of 2,4-D in workers, especially farmers and herbicide applicators, and in members of the general population. Providing detailed information from the extensive number of studies available is beyond the scope of this document, but pertinent data have been extracted from recent reviews (Burns and Swaen 2012; von Stackelberg 2013). Additional information on this topic is presented in Chapter 6.

Burns and Swaen (2012) noted that large studies designed to be representative of the United States (CDC 2009; population surveyed years 1999–2002) and Canadian (Health Canada 2010) populations (surveyed years 2007–2009) did not detect 2,4-D at the 50th percentile (<1 µg/L) (in 50% of the samples, the concentration of 2,4-D was below 1 µg/L of urine). In general, urinary levels of 2,4-D in groups of individuals considered bystanders varied from less than the limit of detection (0.2 µg/L) to 3 µg/L. Bystanders were individuals who did not mix, load, or apply 2,4-D, but who occasionally could have experienced greater exposure than the general population. These included spouses and children of applicators, and applicators of other herbicides. Levels of 2,4-D in the urine from individuals who experienced direct exposures, such as those who applied 2,4-D on crops, forests, and turf, as well as those involved in the manufacture of 2,4-D, varied greatly. Geometric means between 5 and 45 µg/L were reported for crop and forestry applicators; maximum levels varied from 410 to 2,500 µg/L 2,4-D among these groups. A highest maximum of 12,963 µg/L was reported in a study of German manufacturers in the 1980s (Knopp 1994). The wide ranges reported are not surprising considering the number of factors that can determine the extent of exposure, including type of application method, glove use, repairing equipment, size of the area treated, and personal hygiene practices. A study reported that these factors explained 16% of the between-worker variance and 23% of the within-worker variance of urinary 2,4-D concentrations (Bhatti et al. 2010), suggesting that other determinants remained unexplained. It is worth noting that urinary pH is an important determinant of 2,4-D urinary levels (see Section 3.11.2).

Knowing the urinary levels of 2,4-D is important to determine whether someone has been exposed to excessive amounts of 2,4-D. This information is particularly useful if it can be used to estimate an absorbed dose of 2,4-D that can be compared to exposure guidance values. For example, Mage et al. (2004) collected data on urinary creatinine concentration and excretion rate from 978 volunteer participants in the National Health and Nutrition Examination Survey (NHANES), 1988–1994, computed for their age, gender, height, and weight and determined that none of the subjects were exposed to 2,4-D at a rate above the reference dose (RfD) of 5 µg/kg/day established by EPA (EPA 2005a). A number of assumptions were made in this exercise, including assuming that the subjects had a relatively constant intake of 2,4-D and a constant dietary intake of red meat, and that the tubular secretion transport mechanism was not saturated. Under these conditions, the body would excrete approximately constant amounts of 2,4-D and creatinine per day. A similar approach was used by Alexander et al. (2007) to estimate systemic doses in farm families using urine samples collected from the application day through the third day after application. Subjects were participants in the Farm Family Exposure Study, a study of licensed applicators in Minnesota and South Carolina. The geometric means systemic doses (µg/kg/day) were as follows: 2.46 for applicators, 0.8 for spouses, 0.22 for children (all ages included), 0.32 for children 4–11 years of age, and 0.12 for children ≥12 years of age. Exposure to family members was determined primarily by the potential for direct contact with the application process or chemical, although for many spouses and most children, it is more likely to be due to indirect exposure (contamination of surfaces, drift from application areas, in household dust) than direct exposure. Some factors found to be predictive of exposure were use of gloves, size of application, and having to repair equipment. The estimated systemic dose for applicators is consistent with a value of 2.7 µg/kg/day estimated for applicators in a study of participant in the AHS (Thomas et al. 2010b). Scher et al. (2008) developed a simple pharmacokinetic model from 2,4-D urinary excretion data from the Farm Family Exposure Study to evaluate the feasibility of reconstructing absorbed dose of 2,4-D. The model was a one-compartment model with single first-order absorption and elimination rate constants that adequately described the pharmacokinetic disposition of 2,4-D in humans as reported in studies with volunteers (Feldmann and Maibach 1974; Harris and Solomon 1992; Kohli et al. 1974; Sauerhoff et al. 1977). The final analysis was conducted on data from 14 farmers, and the results showed that the model accurately simulated measured urinary output and adequately described the data at early and late time points.

More recent studies have examined the use of biomonitoring equivalents to assess whether exposure to 2,4-D exceeds levels of concern (Aylward et al. 2010; Hays et al. 2012). Studies included both general population adults and children as well as farmers and farm family members. Biomonitoring equivalents

are defined as a concentration of a chemical or its metabolite in a human biological medium (usually blood or urine) that is consistent with existent exposure guidance values (i.e., RfDs). The results of these studies showed that current exposures to 2,4-D are below exposure guidance values for 2,4-D.

3.8.2 Biomarkers Used to Characterize Effects Caused by 2,4-D

Adverse effects, including death, have been observed in humans who intentionally or accidentally ingested herbicide formulations containing 2,4-D. Adverse effects were also reported following cases of accidental dermal exposure to 2,4-D. Some reported effects included tachypnea, tachycardia, vomiting, leukocytosis, liver and kidney congestion in fatal cases, metabolic acidosis, and neurological effects characterized by sensory and motor abnormalities. None of these conditions is specific for 2,4-D; any of these effects or combination of them can be caused by exposure to other chemicals or can be due to conditions unrelated to chemical exposures.

3.9 INTERACTIONS WITH OTHER CHEMICALS

Limited information was located regarding interactions of 2,4-D with other chemicals. 2,4-D was found to increase the expression of some CYP1 cytochromes in rat liver, kidney, and mammary gland (Badawi et al. 2000) and of some microsomal enzymes in the liver of mice (Chaturvedi et al. 1991) and rats (Hietanen et al. 1983), and decrease some phase II enzymes in rat liver (Hietanen et al.1983). This suggests that, in general, the toxicity of chemicals that are metabolized by the affected enzymes will increase or decrease depending on whether metabolism produces a reactive intermediate or a detoxification product. In general, in mice, 2,4-D combined with toxaphene seemed to have additive effects regarding microsomal enzyme induction and liver toxicity; the same, but to a lesser extent, occurred with the combination 2,4-D and parathion (Chaturvedi et al. 1991; Kuntz et al. 1990). Given that exposure to 2,4-D could coexist with exposure to other pesticides, more information on potential interactions would be useful.

3.10 POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

A susceptible population will exhibit a different or enhanced response to 2,4-D than will most persons exposed to the same level of 2,4-D in the environment. Factors involved with increased susceptibility may include genetic makeup, age, health and nutritional status, and exposure to other toxic substances (e.g., cigarette smoke). These parameters result in reduced detoxification or excretion of 2,4-D, or compromised function of organs affected by 2,4-D. Populations who are at greater risk due to their

unusually high exposure to 2,4-D are discussed in Section 6.7, Populations with Potentially High Exposures.

No studies of populations unusually susceptible to 2,4-D toxicity were identified in the literature reviewed.

Studies in animals have shown that 2,4-D is eliminated from the body by active secretion into urine by means of an OAT1 carrier. This carrier protein, which is shared by many animal species including humans, was found to be developmentally-regulated in rats, as expression increased 4-fold between PND 5 and 35 in both male and female rats (Buist et al. 2002). If this were the case also in humans, neonates and/or infants could be at a higher risk for 2,4-D toxicity since lower renal clearance of 2,4-D has been associated with increased systemic toxicity of 2,4-D, as it occurs in dogs (Gorzinski et al. 1987).

A study in rats reported that undernourished pups were more vulnerable to the effects of 2,4-D (body weight, organ's weight) than well-nourished pups (Ferri et al. 2003). A later study from the same group of investigators confirmed the results regarding body weight and reported that undernourished pups also may be more vulnerable to the hypomyelinating effect of 2,4-D (Konjuh et al. 2008).

3.11 METHODS FOR REDUCING TOXIC EFFECTS

This section will describe clinical practice and research concerning methods for reducing toxic effects of exposure to 2,4-D. Because some of the treatments discussed may be experimental and unproven, this section should not be used as a guide for treatment of exposures to 2,4-D. When specific exposures have occurred, poison control centers, board certified medical toxicologists, board-certified occupational medicine physicians and/or other medical specialists with expertise and experience treating patients overexposed to 2,4-D can be consulted for medical advice. The following texts provide specific information about treatment following exposures to 2,4-D:

Goldfrank LR, Hoffman RS, Howland MA, et al., eds. 2014. Goldfrank's toxicologic emergencies. 10th ed. Stamford, CT: Appleton and Lange. An electronic version of this text can be accessed at: http://accessemergencymedicine.mhmedical.com/content.aspx?sectionid=65101011&bookid=1163&jumpsectionID=65101214&Resultclick=2.

Shannon MW, Borron SW, Burns MJ, eds. 2007. Haddad and Winchester's clinical management of poisoning and drug overdose. 4th ed. Philadelphia, PA: WB Saunders Company.

Viccellio P, Bania T, Brent J, et al., eds. 1998. Emergency toxicology. 2nd ed. Philadelphia, PA: Lippincott-Raven Publishers.

Additional relevant information can be found in the front section of this profile under QUICK REFERENCE FOR HEALTH CARE PROVIDERS.

3.11.1 Reducing Peak Absorption Following Exposure

The following information was extracted from the books listed above; specific chapters were written by Roberts (2015), Bradberry et al. (2007), and Craig (1998). It is recommended, however, that this information be used along with consultation with a medical specialist with expertise and experience in treating/managing patients with phenoxy herbicide poisoning.

No information specific for 2,4-D was located; however, Roberts (2015) stated that oral activated charcoal may be given if the patients presents within 1–2 hours of ingestion of an herbicide known to cause significant poisoning. Administration of 50–100 g to an adult may be considered in severely poisoned patients within 1 hour of ingestion (Bradberry et al. 2007). In cases of dermal contact, hair and skin should be cleansed to prevent skin absorption (Craig 1998).

3.11.2 Reducing Body Burden

The following information was extracted from the books listed above; specific chapters were written by Roberts (2015), Bradberry et al. (2007), and Craig (1998). It is recommended, however, that this information be used along with consultation with a medical specialist with expertise and experience in treating/managing patients with phenoxy herbicide poisoning.

Management of patients with acute intoxication with 2,4-D is mainly supportive. However, patients with significant poisoning should be monitored for 24–48 hours, preferably in an intensive care unit (Roberts 2015). Patients with severe hypotonia may be unable to use intercostal muscles for ventilation and would benefit from a period of positive pressure mechanical ventilation (Craig 1998). Because 2,4-D is eliminated almost exclusively in the urine, an adequate renal output may optimize renal excretion and reduce renal toxicity from rhabdomyolysis (Roberts 2015). Urinary alkalinization and hemodialysis should be considered in cases of severe poisoning. Increasing urine pH increases clearance of phenoxy herbicides by “ion trapping” of the chemicals. In one case, increasing urine pH from 5.0 to 8.0 increased renal clearance of 2,4-D from 5.1 to 63 mL/minute (Roberts 2015). It was noted that plasma

alkalinization also may limit the distribution of phenoxy compounds from the central circulation by "ion trapping." Roberts (2015) also noted that "Because phenoxy compounds are small and water soluble, and subject to saturable protein binding with large exposures (increasing the free concentration), they are likely to be cleared by extracorporeal techniques. Extracorporeal elimination using resin hemoperfusion, hemodialysis, or plasmapheresis has been studied in a few cases, with clearances approaching 75 mL/minute." Hemodialysis, however, is the preferred treatment in all severe cases because it greatly enhances clearance without the need for urine pH manipulation and the administration of considerable amounts of intravenous fluid to compromised patients (Bradberry et al. 2007).

3.11.3 Interfering with the Mechanism of Action for Toxic Effects

The mechanism(s) of toxic effects of 2,4-D have not been clearly established; therefore, there are no established methods to interfere with the toxic effects of 2,4-D.

3.12 ADEQUACY OF THE DATABASE

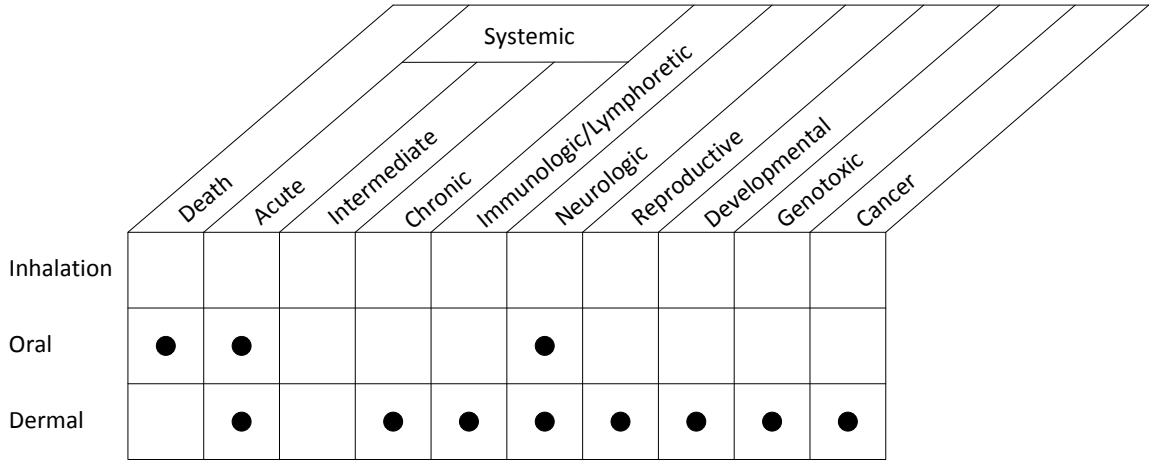
Section 104(I)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of 2,4-D is available. Where adequate information is not available, ATSDR, in conjunction with the National Toxicology Program (NTP), is required to assure the initiation of a program of research designed to determine the adverse health effects (and techniques for developing methods to determine such health effects) of 2,4-D.

The following categories of possible data needs have been identified by a joint team of scientists from ATSDR, NTP, and EPA. They are defined as substance-specific informational needs that if met would reduce the uncertainties of human health risk assessment. This definition should not be interpreted to mean that all data needs discussed in this section must be filled. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

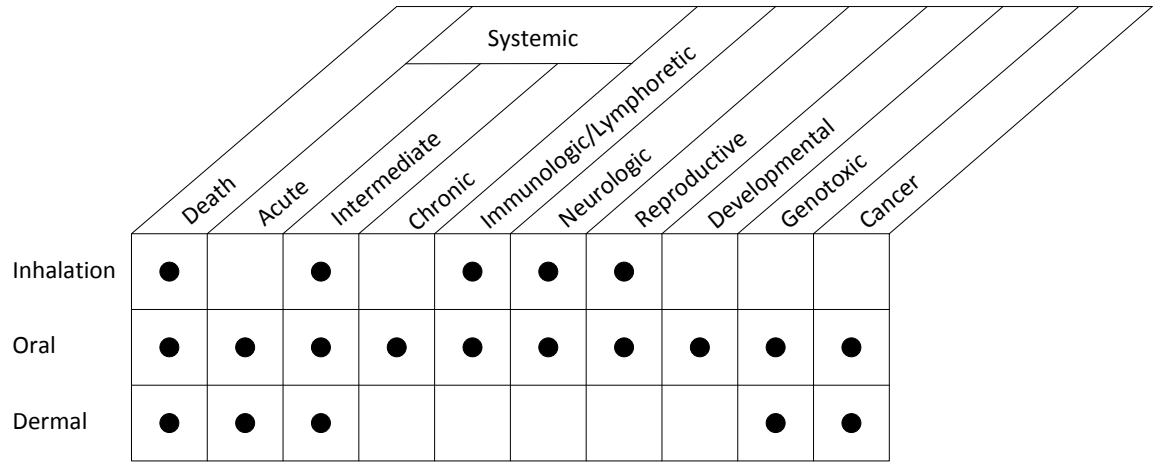
3.12.1 Existing Information on Health Effects of 2,4-D

The existing data on health effects of inhalation, oral, and dermal exposure of humans and animals to 2,4-D are summarized in Figure 3-5. The purpose of this figure is to illustrate the existing information concerning the health effects of 2,4-D. Each dot in the figure indicates that one or more studies provide information associated with that particular effect. The dot does not necessarily imply anything about the

Figure 3-5. Existing Information on Health Effects of 2,4-D



Human



Animal

● Existing Studies

quality of the study or studies, nor should missing information in this figure be interpreted as a "data need". A data need, as defined in ATSDR's *Decision Guide for Identifying Substance-Specific Data Needs Related to Toxicological Profiles* (Agency for Toxic Substances and Disease Registry 1989), is substance-specific information necessary to conduct comprehensive public health assessments. Generally, ATSDR defines a data gap more broadly as any substance-specific information missing from the scientific literature.

Information regarding health effects in humans following exposure to 2,4-D comes from case reports of accidental or intentional ingestion of herbicide formulations containing 2,4-D, accidental skin contact with those products by farmers and professional residential applicators, and occupational exposure during manufacture, formulation, or packaging. Information is also available from exposure of the general population. Exposure to 2,4-D during use of products containing this chemical occurred predominantly by dermal contact, but inhalation may have also occurred if a product was sprayed. The general population can be exposed by dermal contact with surfaces treated with products containing 2,4-D, by consumption of contaminated water or food, and also in house dust. No reliable estimates of quantitative exposure could be obtained from case reports, but studies have estimated exposure from measurements of 2,4-D excreted in the urine. There is no evidence suggesting that the toxicity of 2,4-D is route-specific.

The database in animals is extensive. As can be seen in Figure 3-5, most studies in animals have been conducted by the oral route of exposure. There is more information regarding the health effects of 2,4-D following intermediate-duration exposure than regarding acute- or chronic-duration exposure.

People living near hazardous waste sites may be exposed to 2,4-D primarily via dermal contact with soil contaminated with 2,4-D, through ingestion of contaminated water, or through contaminated house dust.

3.12.2 Identification of Data Needs

Acute-Duration Exposure. No information was located regarding health effects in humans following inhalation exposure to 2,4-D. No acute-duration inhalation studies in animals were located. Published inhalation studies are needed for all exposure durations. There is information regarding health effects in humans following acute-duration oral exposure to 2,4-D from case reports of intentional or accidental ingestion of herbicide formulations containing 2,4-D. Effects that have been reported following oral exposure to high amounts of 2,4-D include including tachypnea, tachycardia, vomiting, leukocytosis, liver and kidney congestion in fatal cases, metabolic acidosis, and death (Dudley and

Thapar 1972; Durakovic et al. 1992; Keller et al. 1994; Nielsen et al. 1965; Smith and Lewis 1987). Because these subjects were exposed to formulations containing 2,4-D along with other ingredients that may have contributed to the effects reported, these studies are inadequate for MRL derivation. Studies in animals provided information on lethality (Drill and Hiratzka 1953; Elo et al. 1988; Gorzinski et al. 1987; Hill and Carlisle 1947) and a wide range of end points including systemic effects (Dickow et al. 2000; Mattsson et al. 1997; Steiss et al. 1987), neurological effects (Mattsson et al. 1997; Steiss et al. 1987; Stürtz et al. 2008), reproductive effects (Dinamarca et al. 2007), and developmental effects (Charles et al. 2001; Chernoff et al. 1990; Collins and Williams 1971; Fofana et al. 2002; Kavlock et al. 1987; Schwetz et al. 1971). An intermediate-duration oral MRL based on developmental effects in rats (Stürtz et al. 2010) was adopted also as acute-duration oral MRL for 2,4-D. Long-term oral studies in animals suggest that the kidney is a target for 2,4-D toxicity; however, virtually no data on kidney effects were available in acute-duration studies. Therefore, an acute-duration study that examines the nature of the dose-response for kidney effects in rats or mice would be useful. Two case reports of humans acutely exposed to products containing 2,4-D by skin contact reported long-lasting neurological alterations (Berkley and Magee 1963; Goldstein et al. 1959). A study in animals with controlled exposure to sublethal doses of 2,4-D would be useful to confirm or refute the reports in humans.

Intermediate-Duration Exposure. No studies of humans exposed to 2,4-D specifically for intermediate-duration periods (15–354 days) were located. However, it is likely that some subjects from studies mentioned below under Chronic-Duration Exposure and Cancer were exposed for intermediate durations. An extensive database in animals exposed by the oral route provided information regarding systemic effects (Bortolozzi et al. 1999; Charles et al. 1996a, 1996c; EPA 1984, 1985, 1986, 1987b; Gorzinski et al. 1987; Marty et al. 2013; Mattsson et al. 1997; Mazhar et al. 2014; Ozaki et al. 2001; Saghir et al. 2013; Stürtz et al. 2010; Troudi et al. 2012a, 2012b), neurological effects (Mattsson et al. 1997; Squibb et al. 1983), reproductive effects (Joshi et al. 2012), and developmental effects (Bortolozzi et al. 1999; EPA 1986; Hansen et al. 1971; Marty et al. 2013; Mazhar et al. 2014; Saghir et al. 2013; Stürtz et al. 2010; Troudi et al. 2012a, 2012b). These studies suggested that the kidney is a target for 2,4-D toxicity. However, because of inconsistencies between studies and uncertainties regarding the toxicological significance of the renal lesions described in some of the reports available for review, this information was not considered for MRL derivation. It would be helpful if the original studies could be obtained to clarify these issues. However, data from a developmental study in rats (Stürtz et al. 2010) were used for deriving an intermediate-duration oral MRL for 2,4-D. A single intermediate-duration inhalation study in animals was available for review (EPA 2008). This study examined a comprehensive number of end points in rats exposed to 2,4-D dusts for 28 days and established a LOAEL of 50 mg/m^3

2,4-D dusts for respiratory effects in rats; a NOAEL was not established. It would be valuable to conduct a study with lower exposure concentrations to establish a NOAEL for respiratory effects. The single study available was considered an insufficient database for MRL derivation. A report summarizing a 21-day dermal study in rabbits provided information mainly on systemic effects (EPA 1991a). A 13-week dermal study in rats or mice would be useful to examine the dose-response relationship for renal effects.

Chronic-Duration Exposure and Cancer. There are numerous studies that provided information regarding exposure to 2,4-D and multiple health outcomes in humans (Beard et al. 2013; Beseler et al. 2006; Bloemen et al. 1993; Bond et al. 1988; Burns et al. 2001, 2011; Cantor et al. 1992; De Roos et al. 2003; Dhillon et al. 2008; Faustini et al. 1996; Eriksson et al. 2008; Flower et al. 2004; Fontana et al. 1998; Garry et al. 1996; Hardell and Eriksson 1999; Hardell et al. 1994; Hartge et al. 2005; Hoar et al. 1986; Hoppin et al. 2006a, 2006b, 2008; Kamel et al. 2006; Kluciński et al. 2001; Kogevinas et al. 1995; Lee et al. 2004; Lerda and Rizzi 1991; McDuffie et al. 2001; Miligi et al. 2006; Mills et al. 2005; Slager et al. 2009; Swan et al. 2003; Tanner et al. 2009; Weisenburger 1990; Weselak et al. 2007, 2008; Yang et al. 2014; Zahm et al. 1990). In these studies, exposure occurred predominantly by the dermal and inhalation routes of exposure. Based on results from these and additional studies, there is no convincing evidence associating exposure to 2,4-D and adverse health effects in humans. As is not uncommon with epidemiological studies, limitations encountered in these studies include unreliable exposure assessment and simultaneous exposures to other chemicals. It seems prudent, however, to continue to monitor populations exposed to 2,4-D, such as pesticide applicators and manufacturers.

Few chronic-duration studies in animals were available for review. These studies provided information on a wide range of end points in rats, mice, and dogs exposed orally to 2,4-D and suggested that the kidney is a target for 2,4-D toxicity in mice (Charles et al. 1996b; EPA 1987a; Hansen et al. 1971). Results from the 2-year study in mice by Charles et al. (1996b) were considered for derivation of a chronic-duration oral MRL for 2,4-D. However, this resulted in a chronic-duration oral MRL for 2,4-D greater than the intermediate-duration oral MRL. Therefore, a chronic-duration oral MRL for 2,4-D was not derived. The chronic-duration oral studies also showed no evidence of carcinogenicity for 2,4-D in rats, mice, or dogs. Additional chronic-duration studies with 2,4-D do not seem necessary at this time.

Genotoxicity. There are data regarding genetic effects in workers exposed to 2,4-D (i.e., Andreotti et al. 2015; Figgs et al. 2000; Garry et al. 2001; Holland et al. 2002; Hou et al. 2013), animals exposed *in vivo* (Amer and Aly 2001; Charles et al. 1999a, 1999b; Epstein et al. 1972; Kaya et al. 1999; Linnainmaa

1984; Madrigal-Bujaidar et al. 2001; Magnuson et al. 1977; Mustonen et al. 1989; Rasmuson and Svahlin 1978; Schop et al. 1990; Tripathy et al. 1993; Venkov et al. 2000; Vogel and Chandler 1974; Yilmaz and Yuksel 2005; Zettenberg et al. 1977), and *in vitro* exposure of prokaryotic cells (Charles et al. 1999a; Garret et al. 1986; Kubo et al. 2002; Mersch-Sundermann et al. 1994; Styles 1973; Venkat et al. 1995; Venkov et al. 2000; Zetterberg 1978; Zetterberg et al. 1977) and eukaryotic cells (Clausen et al. 1990; Galloway et al. 1987; Gonzales et al. 2005; Korte and Jalal 1982; Linnainmaa 1984; Maire et al. 2007; Mikalsen et al. 1990; Mustonen et al. 1986; Soloneski et al. 2007; Turkula and Jalal 1985; Venkov et al. 2000). These studies provided positive and negative results, possibly because of differences in the experimental protocols used by the different studies. Furthermore, unless a population with exposure only to 2,4-D is identified, as in a small group of workers reported by Holland et al. (2002), most studies of farmers or pesticide applicators will provide inconclusive results. However, efforts to design studies to deal with possible confounding should be encouraged.

While there have been studies on the pharmacokinetic profiles for humans (Sauerhoff et al. 1977) and animals (Van Ravenzwaay et al. 2003), it does not appear that much research has been directed towards the 2,4-D conjugate in urine and the potential for reactive oxygen species or other metabolites that may affect hepatic or renal DNA. Studies of this nature are important in establishing a link between metabolism, DNA damage, and potential cancer(s).

Reproductive Toxicity. Three studies of subjects from agricultural communities did not provide convincing evidence suggesting that exposure to 2,4-D is associated with adverse reproductive effects (Arbuckle et al. 2001; Lerda and Rizzi 1991; Swan et al. 2003). Oral studies in animals provided information on gross and microscopic appearance of reproductive organs from males and females (Charles et al. 1996a, 1996c; EPA 1984, 1985, 1986, 1987a; Gorzinski et al. 19897; Hansen et al. 1971) and fertility/reproductive indices (Dinamarca et al. 2007; Hansen et al. 1971; Joshi et al. 2012; Marty et al. 2013; Saghir et al. 2013). These studies suggest that 2,4-D is not a reproductive toxicant. Additional reproductive toxicity studies in animals do not seem necessary at this time.

Results from *in vitro* and *in vivo* studies did not suggest that 2,4-D is an endocrine disruptor chemical (EPA 2015c, 2015d) though some studies describe behavioral effects (Bortolozzi et al. 1998, 1999, 2003; Evangelista de Duffard et al. 1995).

Developmental Toxicity. A few studies are available that examined the potential association between 2,4-D and birth defects and respiratory ailments in children from subjects exposed to 2,4-D

through farming activities (Garry et al. 1996; Sathyanarayana et al. 2010; Weselak et al. 2007, 2008; Yang et al. 2014). The results did not suggest a role for 2,4-D in the health outcomes examined. Studies in animals provide data on standard developmental end points in rodents (Charles et al. 2001; Chernoff et al. 1990; Collins and Williams 1971; EPA 1986; Fofana et al. 2000, 2002; Kavlock et al. 1987; Schwetz et al. 1971; Stürtz et al. 2010), histology of liver and bone from rat pups (Troudi et al. 2012a, 2012b), and neurobehavioral effects in rat pups (Bortolozzi et al. 1999). Some of the studies reported reduced fetal or offspring weight, in many cases accompanied by reduced maternal weight gain during pregnancy or some other maternal effect, and minor soft-tissue and skeletal anomalies, in some studies (Chernoff et al. 1990; Fofana et al. 2000, 2002; Schwetz et al. 1971). 2,4-D did not induce teratogenicity. A study reported a relatively low LOAEL of 2.5 mg 2,4-D/kg/day for reduced body weight in 10-day-old rat pups from dams exposed to 2,4-D on postpartum days 1–16 (Stürtz et al. 2010). This study was used to derive an intermediate-duration oral MRL for 2,4-D. It would be reassuring if other groups of investigators can replicate the findings of Stürtz et al. (2010). Although, as mentioned above, no adverse health outcomes have been reported in children whose mothers were exposed to 2,4-D through farming activities, no information is available regarding levels of 2,4-D in breast milk or in neonates born to these women; pertinent studies would provide useful data.

Immunotoxicity. Two studies of workers exposed to herbicides (2,4-D among them) found no evidence that 2,4-D played a role in minor immunological alterations reported in some workers (Faustini et al. 1996; Kluciński et al. 2001). An epidemiological study did find that male offspring were more prone to allergies (Weselak et al. 2007); however, the pathway for this result has not been studied. De Roos et al. (2005) found no association between rheumatoid arthritis and exposure to 2,4-D among female spouses of participants in the AHS. For the most part, studies in animals have only provided information regarding weight and gross and microscopic appearance of lymphoreticular organs and tissues from rats, mice, and dogs; no significant effects have been reported (Charles et al. 1996a, 1996c; EPA 1984, 1985, 1987a; Gorzinski et al. 1987; Hansen et al. 1971; Marty et al. 2013; Steiss et al. 1987). Only one study monitored parameters of immunocompetence in rats and reported negative results (Marty et al. 2013). 2,4-D was a respiratory allergen in mice sensitized with 2,4-D dermally and challenged with 2,4-D intratracheally (Fukuyama et al. 2009). Conduction of a Tier I screen immunology battery in B6C3F1 mice exposed to 2,4-D would be reassuring.

Neurotoxicity. There is limited information regarding neurological effects from cases of oral or dermal intoxication with commercial products containing 2,4-D (Berkley and Magee 1963; Berwick 1970; Durakovic et al. 1992; Dudley and Thapar 1972; Goldstein et al. 1959). Several studies also

examined the potential association between exposure to 2,4-D and Parkinson's disease (Dhillon et al. 2008; Hancock et al. 2008; Kamel et al. 2006; Tanner et al. 2009). Only Tanner et al. (2009) reported a positive association between 2,4-D and Parkinson's disease. Two studies did not find an association between 2,4-D and depression among female spouses from pesticide applicators in the AHS (Beard et al. 2013; Beseler et al. 2006). Oral studies in animals did not find gross or microscopic alterations in tissues of the nervous system following exposure to 2,4-D (Charles et al. 1996a 1996c; EPA 1984, 1987a; Gorzinski et al. 1987; Hansen et al. 1971; Marty et al. 2013; Mattsson et al. 1997; Squibb et al. 1983; Steiss et al. 1987). A study identified a relatively low LOAEL of 15 mg 2,4-D/kg/day for altered maternal behavior in rats dosed on postpartum days 1–6 (Stürtz et al. 2008). However, the relevance of the alterations (increased latency of retrieval of pups, increased latency of crouching, decreased percent dams licking the pups, decreased percent dams licking the anogenital region of the pups, increased percent of dams leaving the nest, and increased time spent out of the nest) to human health is unknown. The available chronic-duration oral studies did not conduct neurobehavioral tests. Considering that humans may be exposed to low levels of 2,4-D in food items or in drinking water, it would be valuable to determine whether prolonged, low-level exposure to 2,4-D may induce neurobehavioral alterations.

Epidemiological and Human Dosimetry Studies. Many epidemiological studies provided information regarding exposure to 2,4-D and a wide range health outcomes (see Chronic-Duration Exposure and Cancer above for references). Although some studies found that exposure to 2,4-D was positively associated with adverse outcomes, others did not. As previously noted, being significantly associated does not imply causality, although it suggests that exposure to the chemical plays some role in the health outcome assessed and that biological plausibility exists. Conduction of studies in areas where exposures to 2,4-D and other chemicals in the workplace can be adequately characterized would provide valuable information.

Biomarkers of Exposure and Effect.

Exposure. Further refinements to the methodology for estimating exposure levels from urinary levels of 2,4-D, including awareness of factors that can determine the extent of exposure, such as type of application method, glove use, repairing equipment, size of the area treated, and personal hygiene practices, would be valuable. Examining how urine collection timing in relation to exposure can affect the estimates of exposure levels also would be valuable.

Effect. There are no 2,4-D-specific effects following exposure to this substance. Effects that have been associated with acute exposure to high amounts of 2,4-D can also be induced by exposure to other chemicals or can even be caused by conditions unrelated to chemical exposures. Any research aimed at identifying a specific biomarker of effect for 2,4-D would be valuable.

Absorption, Distribution, Metabolism, and Excretion. Information is available regarding absorption, distribution, metabolism, and excretion of 2,4-D in humans and animals following oral and dermal exposure to 2,4-D (Feldmann and Maibach 1974; Griffin et al. 1997a; Harris and Solomon 1992; Khanna and Fang 1966; Kohli et al. 1974; Moody et al. 1990, 1994; Sauerhoff et al. 1977; van Ravenzwaay et al. 2003; Wester et al. 1996). These and additional studies have shown that 2,4-D is almost completely absorbed from the gastrointestinal tract, but dermal absorption is relatively low. 2,4-D distributes widely in tissues following oral exposure, does not accumulate in tissues, is subject to limited metabolism, and is eliminated via the kidneys by a mechanism that involves a saturable carrier protein. The available studies have provided a fairly good characterization of the toxicokinetics of 2,4-D and further studies do not seem necessary at this time.

PBPK models for 2,4-D in rabbits, rats, and humans have been reported (Durkin et al. 2004; Kim et al. 1994, 1995, 1996, 2001). The Kim et al. (1994, 1995, 1996, 2001) and Durkin et al. (2004) models have very different structures, although they appear to yield similar predictions of plasma elimination kinetics when optimized to the same intravenous dosing studies in rats. A particular feature of the Durkin et al. (2004) model is reversible suppression of glomerular filtration and renal blood flow at high 2,4-D concentrations, which results in dose-dependent suppression of urinary excretion. Experimental verification of reversibility of suppression of renal blood flow by 2,4-D would be useful for further validation of this model and its application to human exposures that result in high 2,4-D concentrations.

Comparative Toxicokinetics. Studies in animals have shown the existence of sex and species differences in the toxicokinetics of 2,4-D (Griffin et al. 1997a; Timchalk 2004; van Ravenzwaay et al. 2003). Differences are due principally to the species-dependent activity of the OAT1 carrier protein responsible for the secretion of 2,4-D into the urine. Species with lower capacity to excrete 2,4-D exhibit higher plasma half-life and increased susceptibility to 2,4-D toxicity, as is the case for dogs. Studies of possible genetic determinants of the OAT1 activity carrier in humans could help identify human populations with potentially increased sensitivity to 2,4-D. Studies of OAT1 activity by age, sex, health, and other conditions would be of value to help characterize acceptable exposures for susceptible populations.

Methods for Reducing Toxic Effects. There are no 2,4-D-specific effects following exposure to this chemical. Overexposure to 2,4-D has been associated with tachypnea, tachycardia, vomiting, leukocytosis, liver and kidney congestion in fatal cases, metabolic acidosis, and neurological effects. The mechanisms by which these effects occur have not been elucidated. Management of suspected 2,4-D related toxicity is essentially supportive. Information is available regarding methods that can be used to reduce toxic effects of phenoxy herbicides in general, including gastrointestinal decontamination, hemodialysis, and urinary alkalinization (Bradburry 2007; Roberts 2015). Publishing treatments that have proved to be effective in randomized controlled trials in medical journals could improve and/or prevent secondary effects and speed recovery in the most severe cases.

Children's Susceptibility. Data needs relating to both prenatal and childhood exposures, and developmental effects expressed either prenatally or during childhood, are discussed in detail in the Developmental Toxicity subsection above.

The limited information available regarding effects of 2,4-D in children comes from epidemiological studies of farming communities where 2,4-D has been used and have included monitoring of children These studies have not provided conclusive evidence of associations between 2,4-D and adverse health outcomes in children (Flower et al. 2004; Garry et al. 1996; Metayer et al. 2013; Weselak et al. 2007, 2008; Yang et al. 2014). Continuous monitoring of children exposed to 2,4-D in farming communities is indicated to generate more data.

Animal studies have shown that 2,4-D can be transferred to the offspring through the placenta and via the mother's milk and that it distributes widely in fetal or neonatal tissues (Elo and Ylitalo 1979; Lindquist and Ullberg 1971; Marty et al. 2013; Saghir et al. 2013; Sandberg et al. 1996; Stürtz et al. 2000, 2006). Although there are no reports of 2,4-D in human breast milk, monitoring of women with the highest exposures in farming communities would provide valuable information.

As summarized in Section 3.2.2.6, Developmental Effects, studies in rodents have shown that, for the most part, adverse developmental effects (i.e., mainly reduced body weight in the offspring) occur at maternal dose levels that induced maternal toxicity, mainly reduced maternal weight during pregnancy. Reduced offspring weight was reported in a study in rats administered a relatively low postpartum dose of 2.5 mg 2,4-D/kg/day (Stürtz et al. 2010). Because no such effects have been reported in other studies that exposed dams to considerably higher doses, it would be useful to try to replicate those findings.

Child health data needs relating to exposure are discussed in Section 6.8.1, Identification of Data Needs: Exposures of Children.

3.12.3 Ongoing Studies

The following ongoing research pertaining to 2,4-D was identified in the National Institutes of Health (NIH) Research Portfolio Online Reporting Tools (RePORTER 2015):

Atin Adhikari, from the University of Cincinnati, and coworkers are investigating the potential association between exposure to pesticides (2,4-D among them) among participants in the AHS and atopic immune responses. In the first phase of the research, the investigator will explore immunological activities of unpurified but clinically relevant environmental samples collected in farms (before and after pesticide application) in ovalbumin allergen sensitized mice. The study is sponsored by the National Institute of Environmental Health Sciences (NIEHS).

Laura Beane Freeman, from the Division of Cancer Epidemiology and Genetics of the National Cancer Institute (NCI), and coworkers are investigating potential associations between exposure to pesticides (2,4-D among them) and a wide range of health end points in participants in the AHS. Health end points evaluated include numerous types of cancer, noncancer conditions, and biologic measures. The research is sponsored by the NCI.

Dale Sandler, from the NIEHS, and coworkers are investigating potential associations between exposure to pesticides (2,4-D among them) and health end points among participants in the AHS. The primary focus of the current research is identifying incident cases of respiratory, neurologic, and autoimmune diseases as well as other outcomes reflecting the older age of the cohort. The research is sponsored by the NCI, NIEHS, and EPA.

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4. CHEMICAL AND PHYSICAL INFORMATION

4.1 CHEMICAL IDENTITY

2,4-Dichlorophenoxyacetic acid (2,4-D; Table 4-1) is a free acid, phenoxy herbicide belonging to the phenoxyacetic acid chemical family, which is widely used in the United States. While the free acid is itself used as an herbicide, there are nine forms of 2,4-D registered as active ingredients in end use products. These include salts, amines, and esters of 2,4-D (EPA 2005a). Derivatives include the sodium salt, diethanolamine salt, dimethyl amine salt, isopropylamine salt, triisopropanolamine salt, butoxyethyl ester, ethylhexyl ester, and isopropyl ester (Table 4-2). Almost 90–95% of total 2,4-D global use is accounted for by the dimethyl amine salt and ethylhexyl ester (Charles et al. 2001).

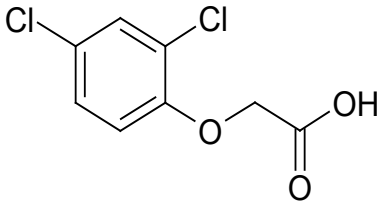
Formulations of 2,4-D and its derivatives vary in their chemical properties and behavior in the environment. However, most quantified analyses of 2,4-D and its derivatives are expressed in terms of the free acid (EPA 2005a).

Information regarding the chemical identity of 2,4-D and its derivatives are provided in Tables 4-1 and 4-2.

4.2 PHYSICAL AND CHEMICAL PROPERTIES

Information regarding the physical and chemical properties of 2,4-D and its derivatives are provided in Tables 4-3 and 4-4.

Table 4-1. Chemical Identity of 2,4-D[a]

Characteristic	Information
Chemical name	2,4-Dichlorophenoxyacetic acid
Synonym(s)	2,4-D; 2,4-D Acid; Acetic acid, (2,4-dichlorophenoxy)-
Registered trade name(s)[b]	Aqua-Kleen; Citrus Fix; Pyresta; Cimarron; Restore; Rush 24; 240; AMINO; Amoxone; Chloroxone; Crop Rider; Dinoxol; Dormone; Emulsamine; Fernimine; Fernoxone; Gesapax-H; Rilof-H; Target; Arena; Campeon; Fenix; Fenix Gold; Stockton; Talion; Turuna; Valsamba; Valsamin; Barrage; Brush-Rhap; Double Up; EndRun; HardBall; Opti-Amine; Trump-Card; Unison; Broadrange; Foundation; Weco Max; Brash; Phenoxy 088; Rugged; Strike; Charge; Dacomin; Chaser; Clean amine; Colt; Crossbow; Rifle; Saber; Salvo; Savage; Shotgun; Whiteout; Defy; Dical; Harvade; Willomine; Duplosan; Dyvel; Lotus; Topshot; U 46; Weedmaster; Speed-Mix; Gen-Amin; Gen-Ester; Grotex Complex; Grox; Trago
Chemical formula	$C_8H_6Cl_2O_3$
Chemical structure	
Identification numbers:	
CAS Registry	94-75-7
NIOSH RTECS	AG6825000[b]
EPA Hazardous Waste	D016; U240
OHM/TADS	No data
DOT/UN/NA/IMDG	UN 3345; UN 3346; UN 3347; UN 3348; IMO 3; IMO 6.1
HSDB	202
NCI	No data

[a]All information obtained from HSDB (2015), unless otherwise noted.
[b]Meister et al. 2014.
[c]RTECS 2009a.

CAS = Chemical Abstracts Services; CIS = Chemical Information System; DOT/UN/NA/IMDG = Department of Transportation/United Nations/North America/International Maritime Dangerous Goods Code; EPA = Environmental Protection Agency; HSDB = Hazardous Substance Data Bank; NCI = National Cancer Institute; NIOSH = National Institute for Occupational Safety and Health; OHM/TADS = Oil and Hazardous Materials/Technical Assistance Data System; RTECS = Registry of Toxic Effects of Chemical Substances

Table 4-2. Chemical Identity of 2,4-D Derivatives

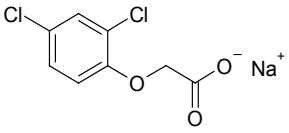
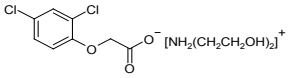
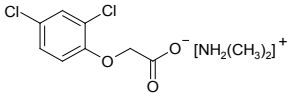
Characteristic	Information[a]		
Chemical name	2,4-D Sodium[b]	2,4-D Diethanolamine[c]	2,4-D Dimethylamine
Synonym(s)	Acetic acid, (2,4-dichlorophenoxy)-, sodium salt; Sodium 2,4-dichlorophenoxy-acetate; 2,4-Dichlorophenoxy-acetic acid, sodium salt; 2,4-D Na[b]	2,4-Diolamine; Acetic acid, (2,4-dichlorophenoxy)-, diethanolamine salt; 2,4-D Bis(2-hydroxyethyl) ammonium; 2,4-D DEA[b,c]	Acetic acid, (2,4-dichlorophenoxy)-, dimethylamine (1:1); (2,4-Dichlorophenoxy) acetic acid dimethylamine salt; Dimethylammonium (2,4-dichlorophenoxy) acetate; 2,4-D DMA
Registered trade name(s)	See 2,4-D in Table 4-1	See 2,4-D in Table 4-1	See 2,4-D in Table 4-1
Chemical formula	$C_8H_5Cl_2O_3.Na$[b]	$C_8H_6Cl_2O_3.C_4H_{11}NO_2$[b]	$C_8H_6Cl_2O_3.C_2H_7N$
Chemical structure[c]			
Identification numbers:			
CAS registry	2702-72-9	5742-19-8	2008-39-1
NIOSH RTECS	No data	No data	No data
EPA hazardous waste	No data	No data	D016; U240
OHM/TADS	No data	No data	No data
DOT/UN/NA/IMDG shipping	No data	No data	UN 3082; UN 3077; IMO 9.0
HSDB	No data	No data	2599
NCI	No data	No data	No data

Table 4-2. Chemical Identity of 2,4-D Derivatives

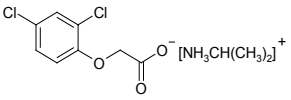
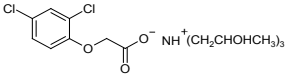
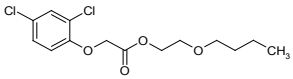
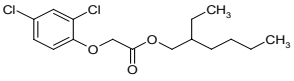
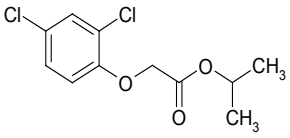
Characteristic	Information[a]		
Chemical name	2,4-D Isopropylamine[c]	2,4-D Triisopropanolamine[c]	2,4-D Butoxyethyl ester
Synonym(s)	2,4-D-isopropylammonium; Acetic acid, (2,4-dichlorophenoxy)-, isopropylamine salt; 2-Propanamine, (2,4-dichlorophenoxy) acetate; 2,4-D IPA[b,c]	Acetic acid, (2,4-dichlorophenoxy)-, triisopropanolamine salt; 2,4-D-tris(2-hydroxypropyl) ammonium; 2-Propanol, 1,1',1''-nitrilotris-, (2,4-dichlorophenoxy) acetate; 2,4-D TIPA[b,c]	Acetic acid, (2,4-dichlorophenoxy)-, 2-butoxyethyl ester; 2,4-Dichlorophenoxy-acetic acid, butoxyethyl ester; 2,4-D BEE
Registered trade name(s)	See 2,4-D in Table 4-1	See 2,4-D in Table 4-1	See 2,4-D in Table 4-1
Chemical formula	$C_8H_6Cl_2O_3.C_3H_9N$[b]	$C_8H_6Cl_2O_3.C_9H_{21}NO_3$[b]	$C_{14}H_{18}Cl_2O_4$
Chemical structure[c]			
Identification numbers:			
CAS registry	5742-17-6	32341-80-3	1929-73-3
NIOSH RTECS	No data	No data	No data
EPA hazardous waste	U240	U240	D016; U240
OHM/TADS	No data	No data	No data
DOT/UN/NA/IMDG shipping	No data	No data	UN 3082; UN 3077; IMO 9.0
HSDB	No data	No data	6307
NCI	No data	No data	No data

Table 4-2. Chemical Identity of 2,4-D Derivatives

Characteristic	Information[a]	
Chemical name	2,4-D Ethylhexyl ester	2,4-D Isopropyl ester
Synonym(s)	Isooctyl(2-ethylhexyl) 2,4-dichlorophenoxy-acetate; 2,4-D, 2-ethylhexyl; 2-Ethylhexyl (2,4-dichlorophenoxy) acetate; Acetic acid, (2,4-dichlorophenoxy)-, 2-ethylhexyl ester; 2,4-D EHE	Acetic acid, (2,4-dichlorophenoxy)-, isopropyl ester; Acetic acid, (2,4-dichlorophenoxy)-, 1-methylethyl ester; 2,4-Dichlorophenoxyacetic acid isopropyl ester; Isopropyl (2,4-dichlorophenoxy)acetate; Isopropyl 2,4-D ester; 2,4-D IPE
Registered trade name(s)	See 2,4-D in Table 4-1	See 2,4-D in Table 4-1
Chemical formula	$C_{16}H_{22}Cl_2O_3$	$C_{11}H_{12}Cl_2O_3$
Chemical structure[c]		
Identification numbers:		
CAS registry	1928-43-4	94-11-1
NIOSH RTECS	No data	No data
EPA hazardous waste	No data	D016; U240
OHM/TADS	No data	No data
DOT/UN/NA/IMDG shipping	No data	UN 3082; UN 3077; IMO 9.0
HSDB	7309	1634
NCI	No data	No data

[a]All information obtained from HSDB (2015), unless otherwise noted.
[b]Meister et al. 2014
[c]EPA 2005a

CAS = Chemical Abstracts Services; CIS = Chemical Information System; DOT/UN/NA/IMDG = Department of Transportation/United Nations/North America/International Maritime Dangerous Goods Code; EPA = Environmental Protection Agency; HSDB = Hazardous Substance Data Bank; NCI = National Cancer Institute; NIOSH = National Institute for Occupational Safety and Health; OHM/TADS = Oil and Hazardous Materials/Technical Assistance Data System; RTECS = Registry of Toxic Effects of Chemical Substances

Table 4-3. Physical and Chemical Properties of 2,4-D[a]

Property	Information
Molecular weight	221.03
Color	White to yellow
Physical state	Crystalline powder
Melting point	138°C
Boiling point	160°C (at 4 mm Hg)
Density/specific gravity:	
at 25°C	1.42
Odor	Odorless; slightly phenolic
Odor threshold	3.13 mg/kg
Solubility:	
Water at 20°C	540 mg/L
Water at 25°C	677 mg/L
Organic solvents at 20°C:	
Ethanol	1,250 g/kg
Diethyl ether	243 g/kg
Heptane	1.1 g/kg
Toluene	6.7 g/kg
Xylene	5.8 g/kg
Octanol	120 g/L (25°C)
Partition coefficients:	
Log K_{ow}	2.81
Log K_{oc}	19.6–135.7
Vapor pressure at 20°C	1.40×10^{-7} mm Hg
Henry's law constant at 20°C	9.75×10^{-8} atm-m^3/mol
Autoignition temperature	No data
Flashpoint	Not combustible
Flammability limits	No data
Conversion factors	No data
Explosive limits	No data

Table 4-4. Physical and Chemical Properties of 2,4-D Derivatives[a]

Property	2,4-D Sodium	2,4-D Diethanolamine
Molecular weight	243.03[b]	326.18[b]
Color	White[b]	Cream[b]
Physical state	Powder[b]	Powder[b]
Melting point	200°C[d]	83°C[d]
Boiling point	No data	No data
Density:		
at 25°C	42.2 pounds/feet^3 (0.676 g/cm^3) (bulk)[d]	0.762 g/cm^3 (bulk)[d]
Odor	No data	No data
Odor threshold:		
Water	No data	No data
Air	No data	No data
Solubility:		
Water at 25°C	4.5x10^4 mg/L (unbuffered solution)[b]	8.06x10^5 mg/L (unbuffered solution)[b]
Organic solvents	No data	No data
Partition coefficients:		
Log K_{ow}	Not applicable[b,c]	0.0224–1.65[b]
Log K_{oc}	No data	No data
Vapor pressure at 25°C	Not applicable[b,c]	9.98x10^{-8} mm Hg[b]
Henry's law constant at 25°C	No data	No data
Autoignition temperature	No data	No data
Flashpoint	No data	No data
Flammability limits	No data	No data
Conversion factors	No data	No data
Explosive limits	No data	No data

Table 4-4. Physical and Chemical Properties of 2,4-D Derivatives[a]

Property	2,4-D Dimethylamine	2,4-D Isopropylamine
Molecular weight	266.1	280.04[b]
Color	White (pure); amber (technical)[b]	Amber[b]
Physical state	Crystals (pure); aqueous liquid (technical)[b]	Aqueous liquid[b]
Melting point	85–87°C	121°C[d]
Boiling point	Decomposition	No data
Density/specific gravity: at 20°C	1.23[d]	1.15[d]
Odor	Odorless	No data
Odor threshold:		
Water	No data	No data
Air	No data	No data
Solubility:		
Water at 25°C	3.0×10^6 g/mL (20°C)	1.74×10^5 g/mL (pH 5) 4.36×10^5 g/mL (pH 7) 3.31×10^5 g/mL (pH 9) (unbuffered solutions)[b]
Organic solvents	Soluble in methyl, ethyl, and isopropyl alcohols, and acetone; insoluble in kerosene and diesel oil	No data
Organic solvents at 20°C		
Acetonitrile	1.06 g/100 mL	
Methanol	>50 g/100 mL	
Toluene	0.165 g/100 mL	
n-Hexane	0.00357 g/100 mL	
Octanol	5.41 g/100 mL	
Partition coefficients:		
Log K_{ow}	0.65	Not applicable[b,c]
Log K_{oc}	1.85–2.13	No data
Vapor pressure at 25°C	1×10^{-7} mm Hg[b]	Not applicable[b,c]
Henry's law constant at 25°C	1.4×10^{-16} atm-m^3/mol[b]	No data
Autoignition temperature	No data	No data
Flashpoint	No data	No data
Flammability limits	Not flammable	No data
Conversion factors	No data	No data
Explosive limits	No data	No data

Table 4-4. Physical and Chemical Properties of 2,4-D Derivatives[a]

Property	2,4-D Triisopropanolamine	2,4-D Butoxyethyl ester
Molecular weight	412.31[b]	321.2
Color	Amber[b]	Amber; colorless
Physical state	Aqueous liquid[b]	Liquid
Melting point	87–110°C[d]	<25°C
Boiling point	No data	89°C[d]
Density/specific gravity: at 20°C	1.21	1.232 g/cm^3
Odor	No data	Odorless (pure); fuel oil-like (technical)
Odor threshold:		
Water	No data	No data
Air	No data	No data
Solubility:		
Water at 25°C	4.61x10^5 g/mL (pH 5) 4.61x10^5 g/mL (pH 7) 1.04x10^5 g/mL (pH 9) (unbuffered solutions)[b]	12 mg/L
Organic solvents	No data	Miscible in acetone, acetonitrile, n-hexane, and methanol; soluble in oils
Partition coefficients:		
Log K_{ow}	Not applicable[b,c]	4.1[b]
Log K_{oc}	No data	No data
Vapor pressure at 25°C	Not applicable[b,c]	4.5x10^{-6} mm Hg
Henry's law constant at 25°C	No data	No data
Autoignition temperature	No data	No data
Flashpoint	No data	>79°C (open cup)
Flammability limits	No data	No data
Conversion factors	No data	No data
Explosive limits	No data	No data

Table 4-4. Physical and Chemical Properties of 2,4-D Derivatives[a]

Property	2,4-D Ethylhexyl ester	2,4-D Isopropyl ester
Molecular weight	333.28	263.12
Color	Golden yellow	Colorless
Physical state	Liquid	Liquid
Melting point	<-37°C	5–25°C
Boiling point	>300°C (decomposition)	240°C[d]
Density:		
at 20°C	1.148	No data
at 25°C/25°C	No data	1.255–1.270
Odor	Sweet, slightly pungent	Fuel oil-like (technical)
Odor threshold:		
Water	No data	No data
Air	No data	No data
Solubility:		
Water at 25°C	0.086 mg/L	37.3 mg/L
Organic solvents	No data	Soluble in alcohols and most oils
Partition coefficients:		
Log K_{ow}	5.78	253.8[d]
Log K_{oc}	No data	2.78[b]
Vapor pressure at 25°C	$3.6x10^{-6}$ mm Hg[b]	$2.32x10^{-4}$ mm Hg
Henry's law constant at 25°C	$1.8×10^{-5}$ atm-m^3/mol	$2.2×10^{-6}$ atm-m^3/mol[b]
Autoignition temperature	No data	No data
Flashpoint	171°C (open cup)	>79°C (open cup)
Flammability limits	No data	No data
Conversion factors	No data	No data
Explosive limits	No data	No data

[a]All information obtained from HSDB (2015), unless otherwise noted.
[b]NPIC 2008.
[c]The salt dissociates to acid in water; therefore, this end point does not apply.
[d]EPA 2005a.

5. PRODUCTION, IMPORT/EXPORT, USE, AND DISPOSAL

5.1 PRODUCTION

2,4-D is an herbicide belonging to the phenoxyacetic acid chemical family (NPIC 2008). It is produced by the reaction of 2,4-dichlorophenolate with monochloroacetic acid or by the reaction between 2,4-dichlorophenol and chloroacetic acid in aqueous sodium hydroxide (HSDB 2015). 2,4-D is sold commercially in the following formulations: emulsifiable concentrate, wettable granules, wettable powder, emulsion (esters), and aqueous solution (salts) (Meister et al. 2014).

Annual production of 2,4-D in the United States was estimated to be 52–67 and 47 million pounds in 1990 and 2001, respectively. Production in the United States was said to be between 50 and <100 million pounds in 2006 according to the EPA's Inventory Update Rule (IUR) (HSDB 2015). The EPA has replaced the IUR with the Chemical Data Reporting (CDR) Rule, which requires manufacturers (including importers) to give EPA nonconfidential information on the chemicals that they manufacture domestically or import into the United States. Data from the CDR lists only one producer of 2,4-D in the United States (the Dow Chemical Company), which declared their production volume as confidential business information for 2012 (EPA 2015f).

2,4-D is a chemical that manufacturing and processing facilities would be required to report under Section 313 of the Emergency Planning and Community Right-to-Know Act (Title III of the Superfund Amendments and Reauthorization Act of 1986 [SARA]) (EPA 2005b). Table 5-1 lists the production year, number of facilities, the state where each facility is located, and the range (in pounds) for each domestic manufacturer that reported production or formulation of 2,4-D in 2014 (TRI13 2015). Manufacturers are required to report Toxics Release Inventory (TRI) data to satisfy EPA requirements. The TRI data should be used with caution since only certain types of facilities are required to report (EPA 2005b). This is not an exhaustive list.

5.2 IMPORT/EXPORT

No current information regarding the import or export of 2,4-D could be located.

Table 5-1. Facilities that Produce, Process, or Use 2,4-D

State[a]	Number of facilities	Minimum amount on site in pounds[b]	Maximum amount on site in pounds[b]	Activities and uses[c]
AR	1	100,000	999,999	9, 12
IA	2	10,000	49,999,999	7
IL	3	1,000	49,999,999	1, 2, 3, 4, 6, 7, 12
IN	1	Not reported	Not reported	Not reported
KS	2	1,000,000	9,999,999	2, 3, 4, 6, 7
MI	1	100,000	999,999	1, 3, 4, 6, 9, 12
MO	1	10,000,000	49,999,999	2, 3, 4, 6, 7
MT	1	1,000,000	9,999,999	2, 3, 6, 7, 9
NE	1	1,000	9,999	12
OH	4	1,000	999,999	7, 12
PA	1	Not reported	Not reported	Not reported
TX	2	1,000	999,999	8, 12
UT	1	10,000	99,999	12
WI	1	Not reported	Not reported	Not reported

[a]Post office state abbreviations used.
[b]Amounts on site reported by facilities in each state.
[c]Activities/Uses:

1. Produce
2. Import
3. Onsite use/processing
4. Sale/Distribution
5. Byproduct
6. Reactant
7. Formulation Component
8. Article Component
9. Repackaging
10. Chemical Processing Aid
11. Manufacturing Aid
12. Ancillary/Other Uses
13. Manufacturing Impurity
14. Process Impurity

Source: TRI13 2015 (Data are from 2013)

5.3 USE

While the free acid is itself used as an herbicide, there are nine forms of 2,4-D registered as active ingredients in end use products. These include salts, amines, and esters of 2,4-D (EPA 2005a). Derivatives include the sodium salt, diethanolamine salt, dimethyl amine salt, isopropylamine salt, triisopropanolamine salt, butoxyethyl ester, ethylhexyl ester, and isopropyl ester. Almost 90–95% of total 2,4-D global use is accounted for by the dimethyl amine salt and ethylhexyl ester (NPIC 2008). 2,4-D and its different chemical forms are listed as an ingredient, either as the singular active ingredient or in conjunction with other ingredients, in about 600 agricultural and residential products (EPA 2005a). The use of 2,4-D ranks first among herbicides in frequency of home and garden applications and third in national herbicide use for agriculture (Gilliom et al. 1999).

2,4-D is sometimes confused with the similarly named chemical, 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), which at one point in time was contaminated with 2,3,7,8-tetrachlorodibenzo-p-dioxin, or TCDD, a confirmed toxin (CDC 2013). However, TCDD has never been a known contaminant of 2,4-D.

2,4-D is used on a wide range of broadleaf and aquatic weeds (EPA 2005a). Registered uses for 2,4-D can be seen in Table 5-2. These uses include application on field, fruit, and vegetable crops, as well as eliminating broadleaf weeds in turf, commercial and residential lawns while not harming the grass, and aquatic and forestry applications. The Midwest, Great Plains, and Northwestern United States have the most 2,4-D usage (EPA 2005a).

2,4-D has been used in the United States since the 1940s (EPA 2005a). Due to some human health concerns, 2,4-D was placed in pre-Special Review by the EPA in 1986. In 1988, it was proposed that Special Review not be initiated due to the lack of epidemiological data linking 2,4-D and carcinogenicity and the final decision was deferred until reregistration. Between 1988 and evaluation for reregistration in 2005, the EPA performed several reviews of epidemiological and other data and still found that none of the new data definitively linked 2,4-D to human cancer cases. In order to address future concerns about its safety, the 2,4-D Industry Task Force agreed to certain changes to labeled uses to reduce exposure. In 2005, the EPA drafted its Reregistration Eligibility Decision (RED) and it was determined that 2,4-D was eligible for reregistration and the final notice not to initiate Special Review was issued (EPA 2005a).

Table 5-2. Registered Uses for 2,4-D

Crop grouping	Representative crops
Terrestrial food crop	Pear, pistachio, stone fruits
Terrestrial food and feed crop	Agricultural fallow/idleland; agricultural rights-of-way/fencerows/ hedgerows; agricultural uncultivated areas; apple; barley; citrus fruits; corn (unspecified); corn, field; corn, pop; corn, sweet; fruits (unspecified); grapefruit; lemon; oats; orange; pome fruits; rice; rye; small fruits; soil, preplant/outdoor; sorghum (unspecified); soybeans (unspecified); sugarcane; tangelo; tree nuts; wheat
Terrestrial feed crop	Grass forage/fodder/hay; pastures; rangeland; rye; sorghum
Terrestrial nonfood crop	Agricultural fallow/idleland; agricultural rights-of-way/fencerows/ hedgerows; agricultural uncultivated areas; airports/landing fields; Christmas tree plantations; commercial/industrial lawns; commercial/institutional/industrial, premises/equipment (outdoor); forest nursery plantings (for transplant purposes); golf course turf; grasses grown for seed; industrial areas (outdoor); nonagricultural outdoor buildings/structures; nonagricultural rights-of-way/fencerows/ hedgerows; nonagricultural uncultivated areas/soils; ornamental and/or shade trees; ornamental lawns and turf; ornamental sod farm (turf); ornamental woody shrubs and vines; paved areas (private roads/sidewalks); potting soil/topsoil; recreation area lawns; recreational area; soil, preplant/outdoor; urban areas
Terrestrial nonfood and outdoor residential	Fencerows/hedgerows; nonagricultural rights-of-way/fencerows/ hedgerows; ornamental and/or shade trees; ornamental lawns and turf; ornamental woody shrubs and vines; paths/patios; paved areas (private roads/sidewalks); urban areas
Aquatic food crop	Agricultural drainage systems; aquatic areas/water; commercial fishery water systems; irrigation systems; lakes/ponds/reservoirs (with human or wildlife use); rice; streams/rivers/channeled water; swamps/ marshes/wetlands/stagnant water
Aquatic nonfood outdoor	Aquatic areas/water; streams/rivers/channeled water; swamps/ marshes/wetlands/stagnant water
Aquatic nonfood industrial	Drainage systems; industrial waste disposal systems; lakes/ponds/ reservoirs (without human or wildlife use)
Forestry	Conifer release; forest plantings (reforestation programs) (tree farms, tree plantations, etc.); forest tree management/forest pest management; forest trees (all or unspecified); forest trees (hardwoods, broadleaf trees); pine (forest/shelterbelt)
Outdoor residential	Residential lawns
Indoor nonfood	Commercial transportation facilities-nonfeed/nonfood

Source: EPA 2005a

The total annual use of 2,4-D in the United States was approximately 46 million pounds, based on data collected from 1992 through 2000. Agricultural use accounted for 66%, or 30 million pounds, while non-agricultural use accounted for 34%, or 16 million pounds. Broken down into area of use in terms of pounds, total 2,4-D use was distributed in the following pattern: pasture and rangeland, 24%; residential lawn with fertilizer, 12%; spring wheat, 8%; winter wheat, 7%; lawn and garden by lawn care and landscape professionals, 7%; residential lawn without fertilizer, 6%; field corn, 6%; soybeans, 4%, summer fallow, 3%; hay not including alfalfa, 3%, and roadways, 3% (EPA 2005a). Use varies from year to year. The U.S. Geological Survey (USGS) Pesticide National Synthesis Project estimated that approximately 38 million pounds of 2,4-D was applied to crops in 2014, with pasture and hay fields, wheat, soybeans, and corn crops receiving the greatest applications (USGS 2016). The development of genetically modified crops that have an increased tolerance to 2,4-D may cause an increase in the total amount applied annually to crops such as soybeans (EPA 2016). Recently, the EPA granted the registration of a new herbicide named Enlist Duo™ containing 2,4-D choline salt and glyphosate for use on genetically modified corn and soybean crops designed to be resistant to 2,4-D and glyphosate (EPA 2014c).

5.4 DISPOSAL

2,4-D should be disposed of by means in accordance with local regulations, such as incineration (Meister et al. 2014).

2,4-D is known to be degraded by soil microorganisms, and therefore, burial in non-crop areas away from water supplies may be an acceptable method of disposal for small quantities (HSDB 2015). The most environmentally acceptable means for 2,4-D disposal is by incineration. Triple rinsing and draining is used for the decontamination of 2,4-D containers and drums. Chemical treatment involves detoxification with chloride of lime or sodium carbonate. Removal of 2,4-D from water may be achieved through the use of activated charcoal or by coagulation and complete treatment by ozonation (HSDB 2015).

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6. POTENTIAL FOR HUMAN EXPOSURE

6.1 OVERVIEW

2,4-D has been identified in at least 46 of the 1,832 hazardous waste sites that have been proposed for inclusion on the EPA National Priorities List (NPL) (ATSDR 2015). However, the number of sites evaluated for 2,4-D is not known. The frequency of these sites can be seen in Figure 6-1.

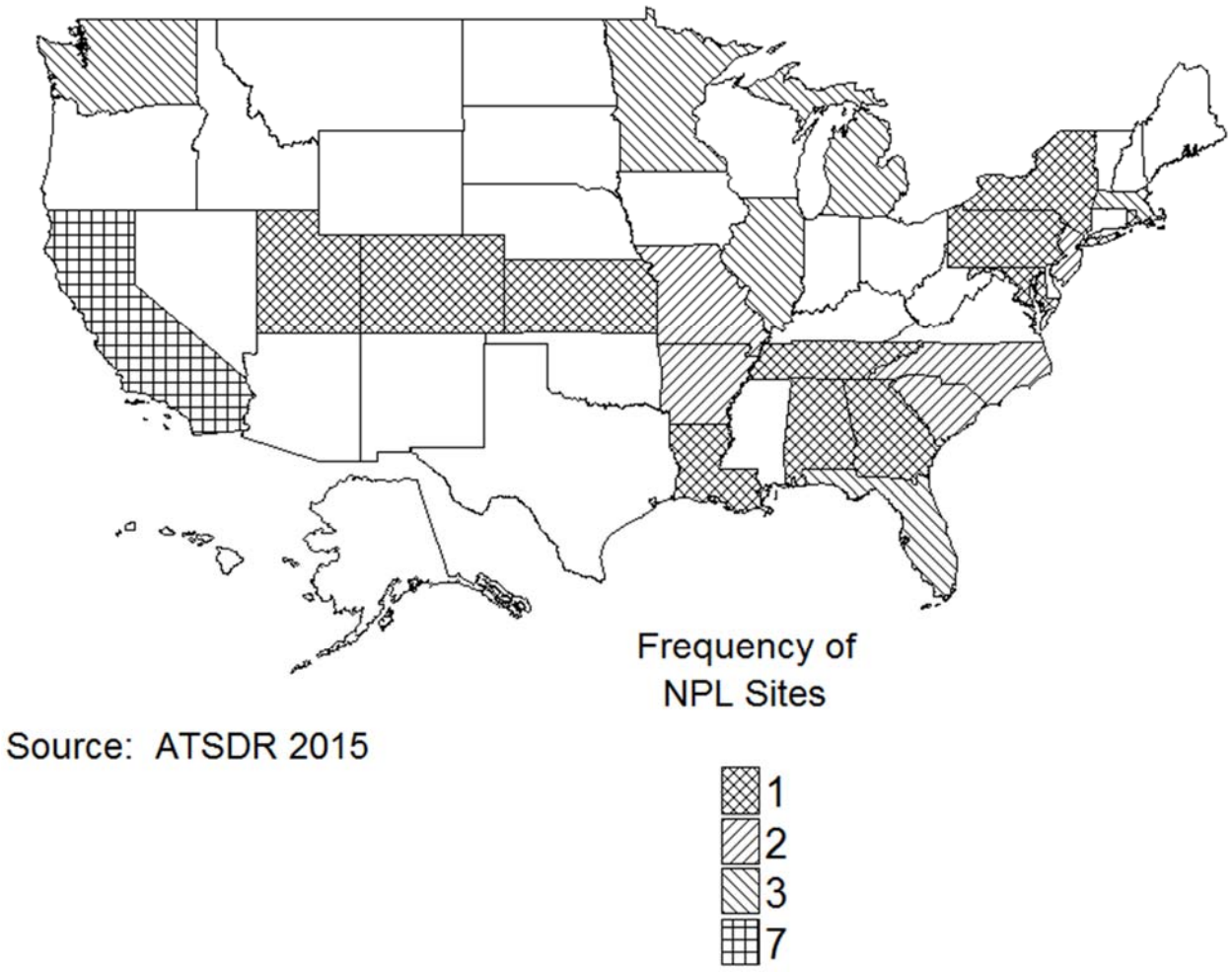
2,4-D is one of the most widely used agricultural herbicides in the United States with approximately 38 million pounds applied to crops in 2014, with pasture and hay fields, wheat, soybeans, and corn crops receiving the greatest applications (USGS 2016). It is also applied to residential or commercial turf for the elimination of a wide variety of broadleaf weeds without causing harm to the grass. Direct applications to rivers or lakes are occasionally made to control certain aquatic plants such as water chestnut or milfoil. Most forms of 2,4-D that are used today are supplied as the dimethyl amine salt (2,4-D DMA) or the ethylhexyl ester (2,4-D EHE).

In the atmosphere, 2,4-D is expected to exist in both the vapor and particulate phase. Vapor-phase 2,4-D is degraded by reaction with photochemically generated hydroxyl radicals with an estimated half-life of about 19 hours (Meylan and Howard 1993). Particulate-phase 2,4,-D is removed from the atmosphere by wet and dry deposition. Atmospheric levels of 2,4-D are generally very low, but detectable levels may be present in agricultural areas where 2,4-D has been applied as an herbicide (WHO 2003).

2,4-D may enter rivers, lakes, and ponds from spray drift following its aerial application or from runoff and erosion of soils treated with 2,4-D. It may also be directly applied to water surfaces in order to eradicate nuisance aquatic plants (Eyres 2009). The aerobic aquatic metabolism half-life of 2,4-D was reported to be about 15 days; however, it was more persistent in anaerobic aquatic metabolism studies, with a half-life ranging from about 41 to 333 days (EPA 2005a). Photolysis in sunlit surface waters may also be an important environmental fate process for 2,4-D, but hydrolysis under environmental conditions is expected to be negligible. Volatilization from water surfaces is not expected to be an important environmental fate process since 2,4-D salts do not volatilize. A bioconcentration factor (BCF) of 1, measured in carp, suggests that bioconcentration in aquatic species is expected to be low (NITE 2010a).

Field dissipation studies conducted in seven states over a 2-year period suggest that 2,4-D is not highly persistent in soils, with half-lives typically ranging from a few days to a few weeks depending upon the

Figure 6-1. Frequency of NPL Sites with 2,4-D Contamination



Source: ATSDR 2015

soil properties, water content of the soil, and whether 2,4-D was applied as a liquid or granular formulation (Wilson et al. 1997). The EPA reported that the biodegradation half-life of 2,4-D in an aerobic mineral soil was 6.2 days and the photodegradation half-life in soil was 68 days (EPA 2005a). Organic carbon normalized soil adsorption coefficients (K_{oc}) values of 70, 76, 59, and 117 using a sandy loam, sand, silty clay loam, and loam soil, respectively, suggest that adsorption to soil surfaces is low (EPA 2005a). Even though 2,4-D is expected to have high mobility in soils, its ability to leach into groundwater may be attenuated by its relatively short half-life in soils.

The general population is exposed to 2,4-D through both its agricultural and residential use. Ingestion of food and water contaminated with small residues of 2,4-D may occur for the general population. Persons residing within or very near areas of heavy 2,4-D use (e.g., farms) would have had an increased risk of exposure to greater amounts of 2,4-D through dermal contact with contaminated plants, soils, or surface waters or by inhalation from the applied herbicide. Those likely to receive the highest exposures are those who are involved in the production, formulation, handling, and application of 2,4-D. Dermal contact appears to be the major route of exposure for workers, although inhalation exposure and accidental ingestion via hand-to-mouth activity is possible. 2,4-D was detected in indoor air and on surfaces (floors, table tops, and window sills) inside single-story Midwestern residences following lawn applications (Nishioka et al. 2001). It was determined that the main transport routes of 2,4-D into the home were from the homeowner applicator and by pets.

6.2 RELEASES TO THE ENVIRONMENT

The Toxics Release Inventory (TRI) data should be used with caution because only certain types of facilities are required to report (EPA 2005b). This is not an exhaustive list. Manufacturing and processing facilities are required to report information to the TRI only if they employ 10 or more full-time employees; if their facility is included in Standard Industrial Classification (SIC) Codes 10 (except 1011, 1081, and 1094), 12 (except 1241), 20–39, 4911 (limited to facilities that combust coal and/or oil for the purpose of generating electricity for distribution in commerce), 4931 (limited to facilities that combust coal and/or oil for the purpose of generating electricity for distribution in commerce), 4939 (limited to facilities that combust coal and/or oil for the purpose of generating electricity for distribution in commerce), 4953 (limited to facilities regulated under RCRA Subtitle C, 42 U.S.C. section 6921 et seq.), 5169, 5171, and 7389 (limited S.C. section 6921 et seq.), 5169, 5171, and 7389 (limited to facilities primarily engaged in solvents recovery services on a contract or fee basis); and if their facility produces,

imports, or processes ≥25,000 pounds of any TRI chemical or otherwise uses >10,000 pounds of a TRI chemical in a calendar year (EPA 2005b).

6.2.1 Air

Estimated releases of 1,264 pounds (~0.57 metric tons) of 2,4-D to the atmosphere from 22 domestic manufacturing and processing facilities in 2014, accounted for about 53% of the estimated total environmental releases from facilities required to report to the TRI (TRI13 2015). These releases are summarized in Table 6-1.

2,4-D is released to the air during application with a wide range of equipment including fixed-wing aircraft, backpack sprayer, band sprayer, boom sprayer, ground directed sprayer, hand held sprayer, helicopter, and tractor-mounted sprayer as well as airblast and chemigation application (EPA 2005a). Available information on the releases of 2,4-D to the air in occupational settings and indoor air, along with exposure levels, is provided in Section 6.5.

6.2.2 Water

Estimated releases of 9 pounds (~0.004 metric tons) of 2,4-D to surface water from 22 domestic manufacturing and processing facilities in 2014, accounted for about 0.38% of the estimated total environmental releases from facilities required to report to the TRI (TRI13 2015). This estimate includes releases to waste water treatment and publicly owned treatment works (POTWs) (TRI13 2015). These releases are summarized in Table 6-1.

2,4-D may enter the aquatic environment through direct application to water for weed control, disposal of wastes from manufacturing and production plants, runoff from treated lands, and drift from application (Sikka et al. 1976).

In 1969, a monitoring program of the irrigation water in the Columbia Basin in Washington reported that the 2,4-D application rate on canal bank weeds ranged from 1.4 to 2.5 pounds per acre (lbs/A) (1.57–2.8 kg/hectare) for a distance of up to 5.1 miles (Bartley and Hattrup 1970). During April–June 1969, about 170,000 gallons of 2,4-D (dimethyl amine salt) was applied to over 18,000 surface acres of Nickajack and Guntersville Reservoirs in Tennessee (Wojtalik et al. 1971). 2,4-D is used to treat aquatic waterbodies for the invasive European water chestnut (*Trapa natans L.)* and Eurasian water milfoil; this likely accounts for most of the intentional releases of this substance to surface waters. For example, in

Table 6-1. Releases to the Environment from Facilities that Produce, Process, or Use 2,4-D[a]

		Reported amounts released in pounds per year[b]							
							Total release		
State[c]	RF[d]	Air[e]	Water[f]	UI[g]	Land[h]	Other[i]	On-site[j]	Off-site[k]	On- and off-site
AR	1	0	0	0	0	0	0	0	0
IA	2	500	0	0	0	0	500	0	500
IL	3	43	0	0	9	0	43	9	52
IN	1	0	0	0	0	0	0	0	0
KS	2	10	0	0	176	0	10	176	186
MI	1	110	9	0	49	0	168	0	168
MO	1	179	0	0	687	0	179	687	866
MT	1	10	0	55	0	0	10	55	65
NE	1	6	0	0	0	0	6	0	6
OH	4	262	0	0	3	110	262	113	375
PA	1	0	0	0	0	0	0	0	0
TX	2	30	0	0	0	0	30	0	30
UT	1	115	0	0	0	0	115	0	115
WI	1	0	0	0	0	0	0	0	0
Total	22	1,264	9	55	924	110	1,323	1,040	2,363

[a]The TRI data should be used with caution since only certain types of facilities are required to report. This is not an exhaustive list. Data are rounded to nearest whole number.
[b]Data in TRI are maximum amounts released by each facility.
[c]Post office state abbreviations are used.
[d]Number of reporting facilities.
[e]The sum of fugitive and point source releases are included in releases to air by a given facility.
[f]Surface water discharges, waste water treatment-(metals only), and publicly owned treatment works (POTWs) (metal and metal compounds).
[g]Class I wells, Class II-V wells, and underground injection.
[h]Resource Conservation and Recovery Act (RCRA) subtitle C landfills; other onsite landfills, land treatment, surface impoundments, other land disposal, other landfills.
[i]Storage only, solidification/stabilization (metals only), other off-site management, transfers to waste broker for disposal, unknown
[j]The sum of all releases of the chemical to air, land, water, and underground injection wells.
[k]Total amount of chemical transferred off-site, including to POTWs.

RF = reporting facilities; UI = underground injection

Source: TRI13 2015 (Data are from 2013)

the summers of 2006, 2007, and 2008, 2,4-D was applied at a rate of 150, 200, and 200 lbs/A, respectively, to a 40-acre wetland in Oneonta, New York in close proximity to the Susquehanna River in order to eradicate overgrowth of water chestnut in this water body (Eyres 2009). 2,4-D formulations (Navigate®, Aquacide® and AquaKleen®) were also applied to a lake in East Haddam, Connecticut between 1999 and 2001 to control milfoil (Bugbee et al. 2003). Most states have strict use guidelines on using 2,4-D in aquatic environments and may require the use of a permit from the state's department of environmental conservation in order to apply these formulations to water bodies. The maximum 2,4-D (acid equivalent) rate for aquatic uses on submerged aquatic plants set by the EPA is 10.8 pounds/acre foot (EPA 2005a).

Effluent samples collected from 52 of the largest municipal wastewater treatment plants and water pollution control facilities in Oregon contained 2,4-D in 3 of 102 samples at a median concentration of 1,630 ng/L and a maximum concentration of 1,890 ng/L in 2010 (Hope et al. 2012).

6.2.3 Soil

Estimated releases of 924 pounds (~0.42 metric tons) of 2,4-D to soils from 22 domestic manufacturing and processing facilities in 2014, accounted for about 39% of the estimated total environmental releases from facilities required to report to the TRI (TRI13 2015). An additional 55 pounds (~0.02 metric tons), constituting about 2.3% of the total environmental emissions, were released via underground injection (TRI13 2015). These releases are summarized in Table 6-1.

More than 3.8 million kg (8.4 million pounds) of 2,4-D were applied to cereal crops in the three prairie provinces (Alberta, Saskatchewan, and Manitoba) of Canada in 1990 (Waite et al. 2002).

The rate per application and rate per year for 2,4-D (acid equivalent) are typically <1.5 and 2.0 pounds/acre/year, respectively (EPA 2005a). The maximum rate for asparagus, forestry uses, and non-cropland uses is 4.0 pounds/acre/year.

Because of its rapid biodegradation in soil, 2,4-D is not likely to be found in soil, except possibly near point sources after immediate release.

6.3 ENVIRONMENTAL FATE

The dominant process affecting the overall environmental fate of 2,4-D is degradation by microbiological activity (Wilson et al. 1997).

6.3.1 Transport and Partitioning

Based on the vapor pressure of 2,4-D (see Table 4-3), 2,4-D released to the atmosphere via spraying applications would be expected to exist in both the vapor and particulate phases (Bidleman 1988).

2,4-D is released to water both from direct application for weed control, and through unintentional processes such as spray drift and runoff. Volatilization is not expected to be significant from water since most formulations of 2,4-D are as salts, which do not volatilize. 2,4-D released to water is not expected to be adsorbed to soils and sediments based on its organic carbon partition coefficient (K_{oc}) values (EPA 1980, 2005; Rao and Davidson 1982; USDA 2001).

Bioaccumulation in aquatic organisms is not expected to be significant, based on a measured bioconcentration factor (BCF) of one for carp (*Cyprinus carpio*) exposed to 1 mg/L of 2,4-D for 28 days (NITE 2010a). Daphnid (*Daphnia magna*, a sand flea) and channel catfish (*Ictalurus melas*) exposed to 0.01 ppm 2,4-D over a period of 4 days had measured depuration half-lives of 13.8 hours and 1.32 days, respectively (Ellgehausen et al. 1980). Rodgers and Stalling (1972) performed a study in which fed and fasted bluegills and channel catfish were exposed to 1.0 mg/L of ^{14}C-labeled 2,4-D butoxyethanol ester for up to 120 hours. Fed channel catfish and bluegills contained 7.3 and 7.8 µg/g (whole body) of 2,4-D after 1 hour of exposure. These levels decreased to 0.04 and 0.45 µg/g, respectively, after 24 hours of exposure, suggesting that uptake and elimination are rapid, but the rates are different for the two species of fish. Similar trends were observed in the fasted fish. Whole-body levels of 9.03 and 16.67 µg/g of 2,4-D after 1 hour of exposure were observed for catfish and bluegills, respectively. These levels increased to 15.74 and 54.55 µg/g, respectively, after 6 hours and then declined to 1.20 and 7.50 µg/g, respectively, after 24 hours, indicating differential uptake and elimination rates between the species of fish. The slower elimination rate of 2,4-D in bluegills versus the channel catfish was further evidenced by the examination of 2,4-D residues in certain organisms of the fish. For example, blood samples of bluegills contained 20.9 µg/g after 8 hours of exposure, whereas catfish contained only 0.1 µg/g; liver samples of catfish contained 0.5 µg/g, while liver samples of bluegills contained 37.6 µg/g after 8 hours.

Bioaccumulation factors of 6 and <10 were reported for exposure to 50 µg/L 2,4-D in algae after 24 hours in a static system and in golden orfe (a fish) after 3 days, respectively (Freitag et al. 1982). Three seaweed species, *Ulva* sp., *Enteromorpha* sp., and *Rhodymenia* sp., exposed to 25 ppb of 2,4-D had a measured uptake of 0.01–0.03% after 24 hours of exposure (Sikka et al. 1976).

2,4-D released to soil partitions to surface water via runoff and to groundwater as a result of leaching. Volatilization of 2,4-D from moist and dry soils is not expected to be a significant transport process. 2,4-D ethylhexyl ester (2,4-D EHE) applied to a sandy loam at a rate of 15.8 lbs/acre was not volatile (<0.22% of the initial amount volatilized) over the course of a 30-day experiment (EPA 2004). It was observed that 2,4-D EHE rapidly transformed to 2,4-D (half-life 8 days), which is expected to exist as an anion under environmental conditions, and anions do not volatilize.

The mobility of 2,4-D in soils and sediments is expected to be high based on measured organic carbon corrected soil adsorption coefficient (K_{oc}) values. An average K_{oc} value of 19.6 was reported in nine soils tested (Rao and Davidson 1982). EPA (1980) measured an average K_{oc} of 109.1 in three soils (a silty clay loam, a sandy clay loam, and fine sand) with a range of 72.2–135.7. This study also reported that as the concentration of 2,4-D in the soil solution phase increased, the mobility increased. The ARS Pesticide Property Database lists K_{oc} values for 2,4-D ranging from 20 to 79 (USDA 2001). K_{oc} values of 70, 76, 59, and 117 were measured using a sandy loam, sand, silty clay loam, and loam soil, respectively (EPA 2005a). Despite the relatively low soil adsorption coefficients of 2,4-D, field dissipation studies have typically indicated only moderate leaching to lower soil levels due to the relatively rapid rate of degradation of 2,4-D (EPA 2004, 2005; Wilson et al. 1997).

2,4-D usually exists as an anion in the environment based its pKa of 2.73 (USDA 2001). Anionic compounds generally adsorb less than their neutral forms to clay or soils with organic carbon (Doucette 2000). Vasudevan and Cooper (2004) showed that soil mineralogy (iron and aluminum oxide content) and exchangeable aluminum content had a direct relationship with the adsorption of anionic 2,4-D, while soil phosphate content had an inverse effect, suggesting that 2,4-D will be more easily leached in soils subject to continued phosphate fertilization and liming. Soil pH also has an effect on mobility. In a study of four soils from rice-producing areas of Arkansas at pH 5 and 7, the mean adsorption coefficient (Kd) of 2,4-D ranged from 0.06 to 0.59 L/kg, and demonstrated that sorption was greatest and mobility was lowest at lower pH, as more of the substance would exist as the fully protonated acid rather than the conjugate base (Johnson et al. 1995).

6.3.2 Transformation and Degradation

Degradation of 2,4-D is primarily by microbiological activity (Wilson et al. 1997). 2,4-D has been shown to undergo degradation in pure cultures by particular species of aerobic microorganisms. The two main pathways of degradation break apart bonds and transform the molecule, creating a hydroxyphenoxy acetic acid intermediate or by acting upon the corresponding phenol (WHO 1989). Half-lives for 2,4-D range from 1.8 to 3.1 days via degradation with a mixture of activated sludge, soil, and sediment microorganisms (Liu et al. 1981).

6.3.2.1 Air

A structure estimation method (Meylan and Howard 1993) was used to approximate a 19-hour half-life for the reaction of 2,4-D with hydroxyl radicals based on a vapor phase reaction rate constant of $6.6x10^{-12}$ cm^3/molecule-second at 25°C. 2,4-D may be susceptible to photolysis by direct sunlight, based on an ultraviolet maxima in the 280–290 range for phenoxy herbicides in aqueous media (HSDB 2015).

6.3.2.2 Water

2,4-D, present at 100 mg/L, reached 0% of its theoretical biological oxygen demand (BOD) in 4 weeks using an activated sludge inoculum at 30 mg/L in the Japanese Ministry of International Trade and Industry (MITI) test (NITE 2010b). However, in other studies, 2,4-D was shown to degrade significantly in sewage sludge. More than 90% of 2,4-D at a concentration of 10–100 ng was mineralized in sewage after 28 days (Subba-Rao et al. 1982). Rosenberg and Alexander (1980) reported that nearly all 2,4-D applied to municipal sewage was degraded after 7 days, and that further additions of 2,4-D were degraded without a lag period.

Radiolabeled 2,4-D at an initial concentration of 4.63 μg/g had a first-order degradation half-life of 15 days using a sediment and water mesocosm maintained under aerobic conditions (EPA 2004). Soluble degradation products identified in the study were chlorohydroquinone and 2,4-dichlorophenol (DCP).

Nesbitt and Watson (1980) showed that the rate of degradation of 2,4-D in river water was directly related to the sediment load and the nutrient concentration; however, the addition of organisms capable of degradation had no effect. 2,4-D incubated in sediment and unfiltered river water obtained during flood conditions degraded quickly with and without the addition of nutrients, which suggests that the water already possessed high phosphorous and nitrogen levels capable of sustaining microbial populations that

degrade 2,4-D. This study reported ranges of half-lives of 2,4-D in river water from 18 to >50 days for clear water with low nutrient loadings and from 10 to 25 days for muddy (nutrient and sediment rich) water obtained after heavy rainfall and flooding conditions with lag times of 6–12 days.

In natural lake water, the extent of mineralization of 2,4-D was reported as 72% in 50 days and was shown to be enhanced by levels of both organics (62.7–95.8% mineralization) and inorganics (84% mineralization) in the water (Wang et al. 1984). Mineralization was also shown to be more rapid at higher concentrations of 2,4-D. This was demonstrated in another study that reported 75–90% mineralization of 2,4-D at concentrations of ≤500 pg/mL in eutrophic lake water in 28 days, but 34% was mineralized at a concentration of 4.9 ng/mL (Subba-Rao et al. 1982).

Preconditioning of organisms to 2,4-D may also increase the rate of degradation. This was shown in a study of the biodegradation of 2,4-D in river water during seasonal variation, which indicated that during different seasons, there was an effect on both 2,4-D concentrations in the water and its degrading capacity (Watson 1977). In these experiments, river water and mud were collected throughout the year from rivers draining from an agricultural region with 2,4-D use and compared to samples collected from rivers draining from forest regions with no recorded 2,4-D use or fertilizer applications. Greater degradation of 2,4-D was observed in the river waters and muds from the agricultural region as compared to the forest region. This was most notable using samples collected after heavy rainfall and flooding conditions where nutrient loadings from fertilizer usage in the agricultural location was common in the runoff into the river. In addition, the soils and waters surrounding the agricultural area with a history of 2,4-D usage is likely to contain greater colonies of microorganisms acclimated to degrading 2,4-D and other herbicides as compared to soils and water from the forest region with no history of herbicide usage. Other factors such as, but not limited to, nutrient load, amount of 2,4-D degrading bacteria, and rainfall amounts are also instrumental in how quickly and how much 2,4-D can be degraded.

2,4-D is stable to hydrolysis (EPA 2005a). In sodium phosphate-buffered waters at pH 2, 7, and 10, there was no observed hydrolysis of 2,4-D, present at 25 µg/L (Chamberlain et al. 2012). Radiolabeled 2,4-D EHE at an initial concentration of 30 µg/L had a first-order half-life of 99.7 days in pH 5 buffer solution, 48.3 days in pH 7 buffer solution, and 52.2 hours in pH 9 buffer solution (EPA 2004).

2,4-D may undergo some degree of photodegradation in surface waters. In a water solution irradiated at 356 nm, 2,4-D had reported photolysis half-lives of 2–4 days (Baur and Bovey 1974). 2,4-D had a half-life of 50 minutes in water irradiated at 254 nm with reaction products 2,4-dichlorophenol,

4-chlorocatechol, 2-hydroxy-4-chlorophenoxyacetic acid, 1,2,4-benzenetriol, and polymeric humic acids (Crosby and Tutass 1966). Furman et al. (2013) studied the photolysis rate of 2,4-D and atrazine in surface water samples collected from agricultural areas in four drainages of the Columbia River Basin in Washington State. They attempted to correlate the photolysis rates with three water quality parameters: nitrate levels in the surface water, dissolved organic carbon levels, and amount of suspended solids in the water samples. An average photolysis rate constant of 0.039/hour was reported for 2,4-D in surface water samples irradiated using a xenon arc lamp, which corresponds to a photolysis half-life of about 18 hours (Furman et al. 2013). Photolysis rates were increased in waters with high nitrate levels as the irradiation of nitrate in surface waters results in the production of hydroxyl radicals, which oxidize 2,4-D and other organic substances. Levels of dissolved organic carbon also showed a positive correlation with the photolysis rate of 2,4-D; however, the levels of suspended solids was inversely proportional to the photolysis rate in the surface water samples at one location. Radiolabeled 2,4-D EHE had a first-order half-life of 128.2 days in pH 5 buffer solution when irradiated with natural sunlight, while a dark control had a half-life of 252.5 days in the pH 5 buffer (EPA 2004). The main photodegradation products were 2,4-D and 2,4-DCP (Furman et al. 2013).

6.3.2.3 Sediment and Soil

2,4-D undergoes biodegradation in soils under most conditions and is not considered persistent. The rate of degradation is affected by nutrient levels, oxygen levels, moisture, temperature, presence of microorganisms, concentration of 2,4-D and whether the soils had previously been acclimated with 2,4-D or other similar herbicides (WHO 1989). Under differing conditions, typical reported half-lives of 2,4-D ranged from <1 day to several weeks (Eder and Weber 1980; Foster and McKercher 1973; Liu et al. 1981; Ou 1984; Rao and Davidson 1982). The EPA Registration Eligibility Decision document for 2,4-D reported that its half-life in an aerobic mineral soil was 6.2 days with several noted metabolites, including 1,2,4-benzenetriol, 2,4-DCP, 2,4-dichloroanisole (DCA), and 4-chlorophenol (EPA 2005a).

Increased moisture, temperature, and organic matter stimulate the degradation of 2,4-D, as demonstrated in a study of the herbicide in two soil types under dry and moist conditions and at two different temperatures (Ou 1984). 2,4,-D was rapidly mineralized using surface soil samples (0–15 cm depth) of a Cecil loamy sand (pH 5.6, 0.9% organic carbon, 6% clay) and a Webster sandy loam (pH 7.3, 3.9% organic carbon, 25% clay) at four different soil moisture levels over a 31-day incubation period and an initial loading rate of about 10 µg 2,4-D per gram of soil (Ou 1984). The half-life of 2,4-D ranged from 3.9 to 9.4 days in the loamy sand and from 7.0 to 253.9 days in the sandy loam depending upon the water

content of the soil at an incubation temperature of 25°C. The greatest degradation rates of 2,4-D occurred for both soils under moist conditions as opposed to dry conditions, suggesting that greater microbial activity occurred in moist as opposed to dry soils and that greater moisture content decreased the amount of bound residues in the soils.

Thirty field dissipation studies conducted in seven states using bare soils and four cropping practices over the 2-year period of 1993–1994 were used to assess the environmental fate of 2,4-D following its application as 2,4-D dimethyl amine salt and 2,4-D EHE with both liquid and granular applications (Wilson et al. 1997). The first set of studies used wheat and turf fields located in Colorado and North Carolina and pastures in Texas. The second set of studies used cornfields from Nebraska and Ohio, wheat fields from North Dakota, and pasture, bare soil and turf fields located in California. Soil half-lives ranged from 1.7 days for turf applications in North Carolina to 12.8 days to pasture fields in Texas during the first set of trials conducted in 1993 in which all applications of 2,4-D were applied as sprays. Half-lives ranged from 2.1 days (bare soil California) to 31.2 days (pasture North Dakota) in the second set of trials conducted in 1994 in which 2,4-D was applied as sprays. Slightly greater half-life ranges were observed for the granular applications as opposed to the liquid sprays, which may be due to the time required to release the herbicides into the soil matrix. Across these studies, <5% of applied 2,4-D leached further than 15 cm from the surface. Moisture content played a major role on the half-life, with higher moisture levels resulting in faster degradation. Since these compounds, and other commercial forms of 2,4-D, are converted rapidly in soil to the same anionic form, these studies were representative of 2,4-D and showed that the chemical form had little effect on the rate of dissipation.

The EPA performed an analysis of the half-life of 2,4-D in various soils depending upon whether it was applied in granular form or as a liquid concentrate (EPA 2004). The granular half-lives ranged from 5.1 to 24.6 days, with a median half-life of 11.9 days, while the concentrate form had half-lives ranging from 1.1 to 42.5 days, with a median half-life of 5.5 days (EPA 2004).

2,4-D EHE was broadcast applied as a spray at a nominal concentration of 4 lbs/acre to a forested sandy clay loam soil located in Georgia (EPA 2004). 2,4-D EHE transformed to 2,4-D, with half-lives of 1.7, 7.2, and 51 days in the protected soil (soil under the forest canopy), foliage, and leaf litter, respectively. 2,4-D EHE was only detected 2 times in exposed soil (not protected by the forest canopy) and was not detected in the exposed soil after 3 days. The half-life of the corresponding 2,4-D was 4 days in the exposed soil, 3.6 days in the protected soil, 23.5 days in foliage, and 52.2 days in the leaf litter (EPA 2004).

2,4-D is generally considered a nonpersistent herbicide; however, at very high application rates, it may be toxic to the microorganisms of some soils or require a prolonged lag period before degradation begins. In a study of 2,4-D applied to various soils representative of the major soil orders of the United States, the lag period and overall degradation rate were directly related to the application rate of 2,4-D (EPA 1980). Formulated and technical-grade 2,4-D degradation, as measured by CO_2 evolution, began around day 10 following applications of 2,4-D at 50 and 500 mg/kg; however, the lag period increased to approximately 21 days at an initial application of 5,000 mg/kg and 50 days at an application rate of 20,000 mg/kg using a Webster silty clay loam soil (EPA 1980). Almost no CO_2 evolution was observed from a sandy loam over the 80-day incubation period at application rates of 5,000 and 20,000 mg/kg, and even the addition of nutrients to the soil did not stimulate degradation.

Preconditioning of organisms to 2,4-D may also increase the rate of degradation in soil. Rosenberg and Alexander (1980) reported 2,4-D added to soil inocula showed 90% degradation after 14 days, after which subsequent additions of 2,4-D was reduced by 70% after 3–4 days. In a long-term field experiment where 2,4-D was applied annually, the complete degradation time was reduced from 10 weeks after one application to 4 weeks after 19 years of annual application (Torstensson et al. 1975).

In a study of the degradation of 2,4-D in soils at different pH levels, the half-life of 2,4-D was 5–8 days in soils in the pH range of 5.0–8.5. Degradation was slower in acidic soils, with half-lives of 21 and 41 days in soils with pH 4.5 and 4.0, respectively (Torstensson 1978).

The half-life of 2,4-D applied to a sterilized soil at 4.31 µg/g and irradiated with sunlight was 68 days (EPA 2004).

6.3.2.4 Other Media

In a study of the degradation of 2,4-D in forest leaf litter from red alder, ceanothus, vine maple, bigleaf maple, or Douglas fir collected in western Oregon, 2,4-D was shown to degrade approximately 25–40% after 15 days (Norris and Greiner 1967).

6.4 LEVELS MONITORED OR ESTIMATED IN THE ENVIRONMENT

Reliable evaluation of the potential for human exposure to 2,4-D depends in part on the reliability of supporting analytical data from environmental samples and biological specimens.

Concentrations of 2,4-D in unpolluted atmospheres and in pristine surface waters are often so low as to be near the limits of current analytical methods. In reviewing data on 2,4-D levels monitored or estimated in the environment, it should also be noted that the amount of chemical identified analytically is not necessarily equivalent to the amount that is bioavailable. The analytical methods available for monitoring 2,4-D in a variety of environmental media are detailed in Chapter 7.

6.4.1 Air

Levels of 2,4-D in the ambient atmosphere are generally low or below the detection limits of the analytical methods used to monitor for this substance, with the exception of areas where it is applied as an herbicide and may reach levels in the low μg/m^3 range. In areas of high use of 2,4-D in Canada, such as cultivated regions, about 40% of air samples collected contained between 0.01 and 0.1 μg/m^3 (WHO 2003). In a monitoring study of the air quality in citrus growing regions of the United States, only 1 of 880 air samples contained 2,4-D at a concentration of 4 μg/m^3 (WHO 2003).

In a study that sampled air from nine locations, both urban and rural, in the United States in 1967 and 1968, 2,4-D was detected in one urban sample in Salt Lake City, Utah at a maximum concentration of 4.0 ng/m^3 (Stanley et al. 1971). 2,4-D was not detected in the air of any of the rural areas sampled, which included locations outside of Buffalo, New York; Dothan, Alabama; Iowa City, Iowa; Orlando, Florida, and Stoneville, Mississippi. During the spraying season in Saskatchewan, Canada in 1972, the 33-day mean daily air concentrations of 2,4-D in urban Saskatoon was 600 ng/m^3, and the 47-day mean daily level was 142 ng/m^3 in Naicam (Que Hee et al. 1975).

In air samples collected in rural south-central Washington at seven and eight stations in 1973 and 1974, respectively, the average 2,4-D concentrations detected were 0.31 and 0.22 μg/m^3, respectively (Farwell et al. 1976). It was reported that the source of 2,4-D was from spray drift from nearby croplands.

In a study of 2,4-D atmospheric levels in an agricultural location in Saskatchewan, Canada where this herbicide was used extensively to treat weed infestations in cereal crops, 2,4-D was detected in 44–63% of the atmospheric samples obtained in the summer of 1989 and 33–53% of the samples obtained in the summer of 1990 (Waite et al. 2002). Mean concentrations ranged from 0.21 to 0.77 ng/m^3 in 1989 and from 0.17 to 0.49 ng/m^3 in 1990. The maximum air concentration of 2,4-D in samples in the summers of 1989 and 1990 was 3.90 ng/m^3 (Waite et al. 2002). 2,4-D detections in 1989 were attributed to atmospheric transport of wind-eroded soils from treated fields in nearby locations since this herbicide had

not been applied near the sampling sites in that summer. The authors also studied the bulk atmospheric deposition of 2,4-D for both of the summers and noted that the highest deposition rates occurred during the month of June, which was the time that the majority of 2,4-D was applied in the region. The maximum bulk deposition rate was 3,550 ng/m^2-day in the summer of 1989 and 1,550 ng/m^2-day in the summer of 1990 (Waite et al. 2002).

2,4-D was detected in indoor air in a study of 13 residences following application to lawn surfaces (Nishioka et al. 2001). No 2,4-D was detected in any indoor air samples 1 week prior to application; however, widespread contamination of both the indoor air and home surfaces (e.g., carpets, floors, etc.) was noted postapplication with notable differences in levels depending upon whether the application was performed by the homeowner or a commercial contractor. Within 2 hours of homeowner application, average 2,4-D levels were approximately 9 and 4 ng/m^3 for PM_{10} and $PM_{2.5}$ associated particle sizes, respectively, and about 4 (PM_{10}) and 1 ($PM_{2.5}$) ng/m^3 following contractor application. By day 3 postapplication, the average levels had decreased to about 3 (PM_{10}) and 1 ($PM_{2.5}$) ng/m^3 in the residences treated by the homeowner and about 2 (PM_{10}) and 1 ($PM_{2.5}$) ng/m^3 in the residences treated by the contractors. The main route of contamination was reported to be track-in practices by the homeowners and their pets.

6.4.2 Water

The widespread use of 2,4-D can result in its occurrence in surface water, groundwater, and drinking water, with concentrations typically in the µg/L range (Botre et al. 2000; USGS 2007).

According to USGS National Water Quality Assessment Program (NAWQA), which monitors groundwater and surface water across the major watersheds in the United States, 2,4-D was one of the most common substances detected in surface water during the 1992–2001 sampling period (USGS 2007). It was detected in roughly 20% of all agricultural streams and 11% of urban streams studied, but was only infrequently detected in undeveloped and mixed land use streams (USGS 2007). Annual maximum concentrations of 2,4-D ranged from 0.003 to 15 µg/L in 4,377 surface water samples obtained from the NAWQA dataset (EPA 2005a).

Over 50% of surface water samples collected from Lakes Ontario, Erie, Huron, and Superior between 1994 and 2000 had detectable concentrations of 2,4-D, with the maximum concentration measured being

0.08 µg/L. The highest concentrations were found near agricultural and urban environments where 2,4-D is used, such as the western basin of Lake Erie (Klecka et al. 2010).

In a study of California surface waters conducted between 2008 and 2011 in three urban areas that included Sacramento (SAC), San Francisco Bay (SFB), and Orange County (OC), 2,4-D was detected in 80–84% of samples collected from SAC and OC, and 66% of samples from SFB (Ensminger et al. 2013). Median concentrations for 2,4-D in SAC, SFB, and OC were approximately 0.4, 0.2, and 0.3 µg/L, respectively. During rainstorm events and increased runoff, the detection frequency and concentration increased. Median concentrations of 2,4-D in the dry season and during a rainstorm were 0.08 and 0.28 µg/L, respectively.

One day after the application of 2,4-D to 7,000 acres in the Loxahatchee National Wildlife Refuge in Florida to control the invasive plant, water hyacinth, at a rate of 4.48 kg/hectare (3.99 lbs/A), the concentration of 2,4-D in surface water in the Hillsboro Canal was 37 µg/L, which decreased to 1–4 µg/L 56 days later (Schultz and Whitney 1974). Eight hours following application of 2,4-D at a rate of 40 lbs/A to the Nickajack and Guntersville Reservoirs in Tennessee to treat invasive Eurasian watermilfoil, levels of about 5,000 µg /L were observed at the water surface and concentrations of 1,500 µg /L were observed at the root depth (Wojtalik et al. 1971). At 2 weeks postapplication, the 2,4-D content was uniformly 650 µg /L and at 1 month postapplication, it was 1 µg /L. Surface water samples collected 4–6 times annually from November 1991 to June 1995 in South Florida had a maximum 2,4-D concentration of 14 µg/L (three detections) (Miles and Pfeuffer 1997). In a study to determine the presence of pesticides in 12 surface water supply intakes in Piedmont and coastal plain regions of North Carolina that were sampled in 1995, 2,4-D was detected in 7% of samples at a concentration range of not detected to 2.42 µg/L (Holman et al. 2000).

From April to September 2007, urban river and stream samples were collected from 19 sites within 16 watersheds, including 15 sites downstream from urban lands, across Canada and analyzed for acidic herbicides (Glozier et al. 2012). 2,4-D concentrations ranged from about 0.010 to 0.60 µg/L. Increased concentrations downstream of urban centers were linked to urban use. In agricultural watersheds sampled in Ontario, Canada from 1981 to 1985, 2,4-D was detected in approximately 9, 6, and 30% of the water samples taken from the mouth of the Grand, Saugeen, and Thames river basins, respectively (Frank and Logan 1988). Mean concentrations of 2,4-D ranged from 0.01 to 0.3 µg/L in the Grand River, from 0.1 to 0.2 µg/L in the Saugeen River, and from 0.3 to 0.7 µg/L in the Thames River (Frank and Logan 1988).

In a 1990 Puget Sound Pesticide Reconnaissance Survey, 15 water samples were collected from five drainage areas that empty into the Puget Sound in Washington and were assessed for pesticide residues (EPA 1991b). 2,4-D was detected in 13 water samples at concentrations ranging from 0.077 to 0.70 µg/L.

Even though 2,4-D is expected to have high mobility in soil, it was detected in <1% of all of the groundwater wells studied from 1992 to 2001 in the NAWQA survey due to its low persistence (USGS 2007). During the NAWQA assessment from 1992 to 1996, in which 2,485 groundwater sites were sampled in 20 of the major hydrologic basins in the United States, 2,4-D was detected in 0.43% of samples, with a maximum concentration of 0.54 µg/L (Kolpin et al. 2000). At 36 U.S. golf courses sampled in 1996, 2,4-D was detected in 8 of 773 groundwater samples at a maximum concentration of 50 µg/L (Cohen et al. 1999). Maximum and mean 2,4-D concentrations of 49.5 and 1.2 µg/L, respectively, were detected in 5 of 50 groundwater samples during a national survey of pesticides in groundwater (EPA 1988).

In the National Contaminant Occurrence Database, 27 of 71 lake/reservoir stations sampled contained a mean dissolved 2,4-D concentration of 0.33 µg/L (range of 0.01–10 µg/L) (EPA 2015e). In 73 of 256 stations where other surface waters were sampled, dissolved 2,4-D was detected at a mean concentration of 0.36 µg/L (range of 0.01–15 µg/L). The mean dissolved 2,4-D in groundwater detected at 5 of 465 stations sampled was reported as 4.0 µg/L (range of 0.01–24 µg/L).

During a study of drinking water supplies in the northern Great Plains of Canada, 15 reservoirs were sampled for pesticides during a spring application period (May to August, 2003) (Donald et al. 2007). 2,4-D was detected in all 206 samples collected, with a maximum reported concentration of 1,850 ng/L (1.850 µg/L). Mean concentrations for reservoirs in Manitoba, Saskatchewan, and Alberta were 46–182, 27–254, and 12–597 ng/L (0.046–0.182, 0.027–0.254, and 0.012–0.597 µg/L), respectively. Atmospheric deposition, snowmelt, and runoff was suspected as the major environmental transport processes responsible for 2,4-D in the reservoirs. The U.S. Department of Agriculture (USDA) Pesticide Data Program (PDP) analyzed 14 groundwater samples from 14 different wells, which included 3 from school/ childcare wells and 11 from private wells in 2013 (USDA 2014). 2,4-D was detected in one sample. Additionally, 2,4-D was detected in 49 of 50 finished drinking water samples at concentrations ranging from 1.1 to 84 ng/L (0.0011–0.084 µg/L) (USDA 2014). It was also detected in 49 of 50 unfinished drinking water samples at concentrations ranging from 1.1 to 99 ng/L (0.0011–0.099 µg/L). Data from the EPA National Contaminant Occurrence Database indicated that 2,4-D was identified at 60 of 415 public water systems derived from surface water sources and 52 of 3,029 public water systems

derived from groundwater at mean levels of 1.18 µg/L (range of 0.1–58 µg/L) and 0.87 µg/L (range of 0.08–8 µg/L), respectively (EPA 2015e).

Rainwater collected between February and October 1996 in Gruze, Switzerland had median and maximum 2,4-D concentrations of 16 and 23 ng/L (0.016 and 0.023 µg/L), respectively (Bucheli et al. 1998).

6.4.3 Sediment and Soil

In soil samples collected from one uncultivated and one cultivated California vertisol soil, 2,4-D concentrations ranged from 8 to 143 ppb at the uncultivated site and was not detected at the cultivated site (Graham et al. 1992). In 13 agricultural soils sampled in Canada between 1987 and 1992, the concentration of 2,4-D ranged from not detected to 38 mg/kg dry weight (Webber and Wang 1995).

In sediment samples collected from Lakes Ontario, Erie, Huron, and Superior from 1994 to 2000, 2,4-D was detected in over 50% of the samples at maximum concentrations of 1.04, 0.74, 0.28, and 0.8 µg/L, respectively (Klecka et al. 2010). Sediment samples taken from the Detroit River and Lake Huron in 1978 contained detectable levels of 2,4-D; however, the concentrations weren't quantified (Konasewich et al. 1978).

6.4.4 Other Environmental Media

During the FDA's Market Basket study that tested 234 ready-to-eat foods 37 times a year between 1982 and 1991, the 10-year average concentration of 2,4-D detected was 0.006 µg/g (Rogers 1995). Levels of 2,4-D in domestic foodstuffs were determined as part of FDA's 2004–2005 Total Diet Studies series (FDA 2005). The food samples were collected between October 2003 and August 2005. 2,4-D was detected in 22 out of 96 food items analyzed for. Twenty-one out of 22 detections were reported at the detection limit of the analytical method. The mean concentrations in µg/g (ppm) reported for 2,4-D in food items were as follows: white, enriched rice, 0.00025; white bread, 0.00060; whole wheat bread, 0.00169; fruit-flavored sweetened cereal, 0.00001; shredded wheat cereal, 0.00012; raisin bran cereal, 0.00035; crisped rice cereal, 0.00006; oat ring cereal, 0.00010; turkey and rice baby food, 0.00004; cracked wheat bread, 0.00098; rice cereal baby food, 0.00003; and meatless, Chinese fried rice, 0.00015. The most frequent detections of 2,4-D were found in bread products (FDA 2005). In 1971, 2,4-D was detected in 7 of 4,638 samples of dairy milk (Duggan et al. 1983).

Following the application of 2,4-D to 7,000 acres in the Loxahatchee National Wildlife Refuge in Florida at a rate of 4.48 kg/hectare (3.99 lbs/A), 2,4-D was detected in the breast muscle and liver of Florida gallinules at concentrations of 0.30 and 0.675 mg/kg, respectively, 1 day after spraying. Four days after spraying, no 2,4-D was detected. In 60 fish sampled, 19 had detectable 2,4-D residues in muscle tissue at concentrations ranging from <0.010 to 0.162 mg/kg (Schultz and Whitney 1974).

After treatment of the Nickajack and Guntersville Reservoirs on the Tennessee River with 2,4-D in 1969, concentrations in plankton 1, 8, and 24 hours and 14, 28, 30, 120, and 160 days after application were 0.06, 0.88, 1.8, 2.6, 3.6, 2.2, 1.1, and 3.7 ppm, respectively (Wojtalik et al. 1971). Whole body concentrations of eight species of freshwater fish from the Guntersville Reservoir did not rise above the pretreatment level of <0.10 mg/kg, with the exception of gizzard shad which had concentrations of 0.34, <0.10, 0.22, and <0.10 mg/kg at 28, 60, 120, and 180 days after application, respectively.

6.5 GENERAL POPULATION AND OCCUPATIONAL EXPOSURE

The general population may be exposed to 2,4-D during and after its use in residential and recreational areas. These include application to residential lawns, golf courses, parks, cemeteries, wooded areas, and other grassy areas. Since 2,4-D is also used on aquatic weeds, swimmers may be exposed when swimming in waters treated with 2,4-D (EPA 2005a). Transport of 2,4-D into residential homes may occur from agricultural spray drift, volatilization, soil or dust resuspension, tracked in on shoes, and on clothing (Nishioka et al. 2001). 2,4-D exposure for the general population is typically at or near the level of detection (CDC 2015). The reported limit of detection values ranged from 0.2 to 20 μg/L in the biomonitoring and epidemiology studies reviewed.

The National Health and Nutrition Examination Survey (NHANES) uses biomonitoring to provide estimates of exposure to the civilian U.S. population. Chemicals and their metabolites are measured in subsets of participants aged 6–59 years old, meant to be a representative sample of the population. Urine measurements are reported as both the concentration in urine and the concentration corrected for urine-creatinine level, which adjusts for urine dilution. Urinary levels of 2,4-D were measured in several NHANES programs assessing exposure to subsets of the general population in the United States from years 1999–2000, 2001–2002, and 2003–2004, 2005–2006, 2007–2008, and 2009–2010 (CDC 2015). For survey years 1999–2000, 2001–2002, and 2007–2008, no geometric mean urinary concentration of the 2,4-D could be calculated because the proportion of results below the detection limit was too high to provide a valid result. The NHANES results for 1999–2010 are summarized in Tables 6-2 and 6-3 (CDC

Table 6-2. Geometric Mean and Selected Percentiles of 2,4-D Urine Concentrations (in µg/L) for the U.S. Population from the National Health and Nutrition Examination Survey (NHANES) 1999–2010

	Survey years	Geometric mean (95% CI)	Selected percentiles (95% CI)				Sample size
			50th	75th	90th	95th	
Total	1999–2000	*	<LOD	<LOD	<LOD	<LOD	1,977
	2001–2002	*	<LOD	0.220 (<LOD–0.310)	0.690 (0.560–0.880)	1.26 (1.01–1.36)	2,903
	2003–2004	0.245 (0.210–0.286)	0.230 (0.180–0.320)	0.580 (0.490–0.660)	1.10 (0.910–1.34)	1.71 (1.41–2.37)	2,488
	2007–2008	*	<LOD	0.550 (0.530–0.590)	1.06 (0.940–1.19)	1.60 (1.38–1.79)	2,587
	2009–2010	0.308 (0.275–0.345)	0.280 (0.250–0.320)	0.530 (0.470–0.600)	0.930 (0.810–1.08)	1.43 (1.12–2.02)	2,747
Age group							
6–11 years	1999–2000	*	<LOD	<LOD	<LOD	1.30 (<LOD–2.40)	477
	2001–2002	*	<LOD	0.310 (0.210–0.400)	0.740 (0.550–1.13)	1.55 (1.00–2.21)	546
	2003–2004	0.266 (0.214–0.332)	0.290 (0.200–0.390)	0.670 (0.440–0.920)	1.03 (0.890–1.40)	1.88 (1.01–2.54)	309
	2007–2008	*	<LOD	0.720 (0.630–0.860)	1.44 (1.15–1.64)	1.93 (1.62–2.84)	385
	2009–2010	0.385 (0.330–0.449)	0.350 (0.290–0.440)	0.670 (0.510–0.780)	1.20 (0.860–1.58)	1.59 (1.36–2.77)	386
12–19 years	1999–2000	*	<LOD	<LOD	<LOD	1.10 (<LOD–1.60)	677
	2001–2002	*	<LOD	0.250 (<LOD–0.420)	0.690 (0.440–1.16)	1.24 (.690–1.66)	797
	2003–2004	0.256 (0.212–0.310)	0.260 (0.180–0.380)	0.580 (0.470–0.710)	1.04 (0.890–1.31)	1.66 (1.20–2.97)	714
	2007–2008	*	<LOD	0.590 (0.530–0.670)	1.29 (0.790–1.97)	2.38 (1.46–2.73)	390
	2009–2010	0.301 (0.248–0.366)	0.280 (0.240–0.330)	0.490 (0.420–0.620)	0.900 (0.660–1.05)	1.12 (0.880–2.88)	401
20–59 years	1999–2000	*	<LOD	<LOD	<LOD	<LOD	823
	2001–2002	*	<LOD	0.210 (<LOD–0.310)	0.690 (0.540–0.910)	1.27 (0.930–1.49)	1,070
	2003–2004	0.239 (0.205–0.279)	0.220 (0.170–0.300)	0.570 (0.480–0.640)	0.980 (0.840–1.35)	1.55 (1.25–2.50)	937
	2007–2008	*	<LOD	0.530 (0.490–0.570)	0.970 (0.800–1.17)	1.36 (1.22–1.78)	1,179
	2009–2010	0.288 (0.259–0.319)	0.270 (0.230–0.310)	0.500 (0.440–0.560)	0.870 (0.740–1.04)	1.33 (1.05–1.69)	1,309

Table 6-2. Geometric Mean and Selected Percentiles of 2,4-D Urine Concentrations (in µg/L) for the U.S. Population from the National Health and Nutrition Examination Survey (NHANES) 1999–2010

	Survey years	Geometric mean (95% CI)	Selected percentiles (95% CI)				Sample size
			50th	75th	90th	95th	
≥60 years	2001–2002	*	<LOD	<LOD	0.560 (0.390–0.870)	1.26 (0.690–1.78)	490
	2003–2004	0.248 (0.205–0.301)	0.210 (0.130–0.320)	0.560 (0.470–0.680)	1.36 (1.07–1.90)	2.42 (1.66–3.67)	528
	2007–2008	*	<LOD	0.560 (0.530–0.640)	1.02 (0.840–1.12)	1.46 (1.10–2.11)	633
	2009–2010	0.349 (0.294–0.414)	0.300 (0.230–0.390)	0.590 (0.510–0.720)	1.11 (0.810–1.57)	2.08 (1.16–5.40)	651
Gender							
Males	1999–2000	*	<LOD	<LOD	<LOD	1.10 (<LOD–1.80)	962
	2001–2002	*	<LOD	0.330 (0.220–0.490)	0.890 (0.690–1.17)	1.49 (1.26–2.03)	1,364
	2003–2004	0.276 (0.240–0.317)	0.290 (0.210–0.370)	0.630 (0.540–0.740)	1.22 (0.960–1.42)	2.12 (1.42–2.73)	1,218
	2007–2008	*	<LOD	0.610 (0.580–0.650)	1.26 (1.05–1.38)	2.11 (1.68–2.41)	1,292
	2009–2010	0.347 (0.298–0.404)	0.320 (0.270–0.370)	0.580 (0.500–0.690)	1.05 (0.810–1.47)	1.82 (1.12–4.14)	1,343
Females	1999–2000	*	<LOD	<LOD	<LOD	<LOD	1,015
	2001–2002	*	<LOD	<LOD	0.470 (0.360–0.620)	0.890 (0.670–1.21)	1,539
	2003–2004	0.219 (0.181–0.264)	0.190 (0.110–0.280)	0.490 (0.400–0.630)	0.980 (0.860–1.33)	1.48 (1.31–2.27)	1,270
	2007–2008	*	<LOD	0.500 (0.460–0.540)	0.870 (0.790–1.01)	1.28 (1.12–1.42)	1,295
	2009–2010	0.275 (0.250–0.303)	0.260 (0.220–0.300)	0.480 (0.440–0.540)	0.860 (0.740–0.950)	1.14 (.970–1.39)	1,404
Race/ethnicity							
Mexican Americans	1999–2000	*	<LOD	<LOD	<LOD	<LOD	695
	2001–2002	*	<LOD	0.250 (<LOD–0.330)	0.730 (0.610–0.890)	1.20 (.960–1.36)	743
	2003–2004	0.313 (0.256–0.383)	0.340 (0.260–0.440)	0.730 (0.610–0.840)	1.42 (1.02–1.52)	1.81 (1.23–3.53)	606
	2007–2008	*	<LOD	0.520 (0.470–0.590)	0.860 (0.790–1.00)	1.46 (0.950–2.22)	500
	2009–2010	0.276 (0.240–0.318)	0.250 (0.210–0.300)	0.470 (0.410–0.570)	0.840 (0.680–1.08)	1.23 (0.830–2.02)	602

Table 6-2. Geometric Mean and Selected Percentiles of 2,4-D Urine Concentrations (in µg/L) for the U.S. Population from the National Health and Nutrition Examination Survey (NHANES) 1999–2010

	Survey years	Geometric mean (95% CI)	Selected percentiles (95% CI)				Sample size
			50th	75th	90th	95th	
Non-Hispanic blacks	1999–2000	*	<LOD	<LOD	<LOD	1.20 (<LOD–1.70)	520
	2001–2002	*	<LOD	<LOD	0.560 (0.420–0.890)	1.06 (0.790–1.48)	743
	2003–2004	*	0.190 (<LOD–0.290)	0.510 (0.380–0.630)	0.910 (0.750–1.22)	1.31 (0.990–1.98)	648
	2007–2008	*	<LOD	0.580 (0.530–0.630)	1.05 (0.910–1.20)	1.49 (1.23–1.97)	574
	2009–2010	0.284 (0.251–0.321)	0.260 (0.240–0.290)	0.460 (0.390–0.540)	0.790 (0.620–1.03)	1.11 (0.790–1.81)	504
Non-Hispanic whites	1999–2000	*	<LOD	<LOD	<LOD	<LOD	589
	2001–2002	*	<LOD	0.240 (<LOD–0.360)	0.730 (0.560–0.980)	1.30 (1.01–1.66)	1,201
	2003–2004	0.254 (0.211–0.306)	0.240 (0.180–0.360)	0.590 (0.470–0.720)	1.17 (0.930–1.41)	2.00 (1.40–2.51)	1,076
	2007–2008	*	<LOD	0.560 (0.540–0.600)	1.12 (0.940–1.29)	1.61 (1.36–2.16)	1,083
	2009–2010	0.328 (0.281–0.382)	0.300 (0.250–0.370)	0.570 (0.480–0.680)	0.980 (0.830–1.20)	1.57 (1.14–2.77)	1,200

CI = confidence interval

Source: CDC 2015

Table 6-3. Geometric Mean and Selected Percentiles of 2,4-D Urine Concentrations (Creatinine Corrected) (in µg/g of Creatinine) for the U.S. Population from the National Health and Nutrition Examination Survey (NHANES) 1999–2010

	Survey years	Geometric mean (95% CI)	Selected percentiles (95% CI)				Sample size
			50th	75th	90th	95th	
Total	1999–2000	*	<LOD	<LOD	<LOD	<LOD	1,977
	2001–2002	*	<LOD	0.378 (<LOD–0.412)	0.700 (0.635–0.778)	1.12 (1.03–1.26)	2,901
	2003–2004	0.241 (0.203–0.287)	0.253 (0.206–0.290)	0.500 (0.423–0.610)	1.03 (0.855–1.28)	1.85 (1.42–2.50)	2,486
	2007–2008	*	<LOD	0.737 (0.667–0.779)	1.28 (1.17–1.40)	1.84 (1.65–2.12)	2,585
	2009–2010	0.321 (0.286–0.360)	0.301 (0.272–0.329)	0.500 (0.458–0.573)	0.983 (0.846–1.19)	1.55 (1.30–2.12)	2,747
Age group							
6–11 years	1999–2000	*	<LOD	<LOD	<LOD	1.32 (<LOD–2.24)	477
	2001–2002	*	<LOD	0.485 (0.378–0.679)	1.13 (0.825–1.35)	1.41 (1.27–1.73)	546
	2003–2004	0.323 (0.249–0.421)	0.320 (0.250–0.440)	0.744 (0.500–1.06)	1.30 (0.990–2.55)	2.55 (1.23–5.16)	309
	2007–2008	*	<LOD	0.970 (0.817–1.24)	1.65 (1.47–1.85)	2.96 (1.65–6.18)	385
	2009–2010	0.521 (0.444–0.610)	0.478 (0.411–0.531)	0.792 (0.674–1.06)	1.52 (1.21–1.74)	2.20 (1.53–3.02)	386
12–19 years	1999–2000	*	<LOD	<LOD	<LOD	0.593 (<LOD–1.05)	677
	2001–2002	*	<LOD	0.275 (<LOD–0.376)	0.483 (0.328–0.662)	0.662 (0.517–0.918)	796
	2003–2004	0.193 (0.160–0.232)	0.205 (0.157–0.250)	0.419 (0.328–0.460)	0.709 (0.540–0.925)	1.23 (0.837–2.35)	713
	2007–2008	*	<LOD	0.555 (0.475–0.651)	0.908 (0.778–1.05)	1.56 (0.950–2.79)	388
	2009–2010	0.258 (0.212–0.314)	0.256 (0.200–0.299)	0.358 (0.320–0.439)	0.706 (0.439–1.05)	1.05 (0.579–3.27)	401
20–59 years	1999–2000	*	<LOD	<LOD	<LOD	<LOD	823
	2001–2002	*	<LOD	0.378 (<LOD–0.412)	0.667 (0.593–0.778)	1.08 (0.806–1.29)	1,070
	2003–2004	0.227 (0.188–0.274)	0.242 (0.196–0.278)	0.452 (0.397–0.545)	0.923 (0.708–1.20)	1.48 (1.14–2.43)	936
	2007–2008	*	<LOD	0.667 (0.588–0.769)	1.17 (1.04–1.34)	1.65 (1.43–2.33)	1,179
	2009–2010	0.288 (0.259–0.321)	0.276 (0.250–0.309)	0.458 (0.418–0.507)	0.860 (0.750–0.962)	1.36 (1.00–1.88)	1,309

Table 6-3. Geometric Mean and Selected Percentiles of 2,4-D Urine Concentrations (Creatinine Corrected) (in µg/g of Creatinine) for the U.S. Population from the National Health and Nutrition Examination Survey (NHANES) 1999–2010

	Survey years	Geometric mean (95% CI)	Selected percentiles (95% CI)				Sample size
			50th	75th	90th	95th	
≥60 years	2001–2002	*	<LOD	<LOD	0.824 (0.583–1.10)	1.34 (1.00–2.16)	489
	2003–2004	0.301 (0.248–0.366)	0.310 (0.237–0.385)	0.657 (0.510–0.866)	1.54 (1.16–1.95)	3.00 (1.95–6.36)	528
	2007–2008	*	<LOD	0.860 (0.781–0.903)	1.53 (1.27–1.72)	1.96 (1.60–2.33)	633
	2009–2010	0.414 (0.356–0.480)	0.354 (0.306–0.407)	0.667 (0.548–0.812)	1.41 (0.983–1.99)	2.87 (1.49–4.49)	651
Gender							
Males	1999–2000	*	<LOD	<LOD	<LOD	0.667 (<LOD–1.16)	962
	2001–2002	*	<LOD	0.336 (0.272–0.412)	0.652 (0.560–0.825)	1.14 (0.979–1.39)	1,364
	2003–2004	0.227 (0.189–0.271)	0.238 (0.194–0.276)	0.473 (0.412–0.564)	0.941 (0.767–1.23)	1.80 (1.09–2.79)	1,217
	2007–2008	*	<LOD	0.596 (0.538–0.670)	1.14 (0.980–1.24)	1.63 (1.47–2.15)	1,291
	2009–2010	0.309 (0.266–0.359)	0.282 (0.242–0.323)	0.481 (0.413–0.554)	1.01 (0.707–1.57)	1.80 (1.06–3.88)	1,343
Females	1999–2000	*	<LOD	<LOD	<LOD	<LOD	1,015
	2001–2002	*	<LOD	<LOD	0.711 (0.631–0.809)	1.10 (0.933–1.26)	1,537
	2003–2004	0.256 (0.213–0.308)	0.263 (0.212–0.311)	0.522 (0.435–0.645)	1.14 (0.900–1.42)	1.85 (1.42–2.64)	1,269
	2007–2008	*	<LOD	0.854 (0.757–0.903)	1.47 (1.23–1.58)	1.91 (1.65–2.33)	1,294
	2009–2010	0.334 (0.302–0.369)	0.319 (0.288–0.355)	0.533 (0.475–0.611)	0.953 (0.862–1.10)	1.40 (1.21–1.55)	1,404
Race/ethnicity							
Mexican Americans	1999–2000	*	<LOD	<LOD	<LOD	<LOD	695
	2001–2002	*	<LOD	0.350 (<LOD–0.386)	0.720 (0.583–0.840)	1.08 (.778–1.56)	743
	2003–2004	0.287 (0.223–0.371)	0.309 (0.194–0.459)	0.593 (0.463–0.771)	1.08 (0.833–1.36)	1.54 (1.17–3.19)	605
	2007–2008	*	<LOD	0.622 (0.571–0.691)	1.15 (0.903–1.43)	1.74 (1.37–2.33)	499
	2009–2010	0.289 (0.255–0.326)	0.282 (0.255–0.300)	0.434 (0.392–0.495)	0.781 (0.565–1.11)	1.30 (.733–2.63)	602

Table 6-3. Geometric Mean and Selected Percentiles of 2,4-D Urine Concentrations (Creatinine Corrected) (in µg/g of Creatinine) for the U.S. Population from the National Health and Nutrition Examination Survey (NHANES) 1999–2010

	Survey years	Geometric mean (95% CI)	Selected percentiles (95% CI)				Sample size
			50th	75th	90th	95th	
Non-Hispanic blacks	1999–2000	*	<LOD	<LOD	<LOD	0.593 (<LOD–1.19)	520
	2001–2002	*	<LOD	<LOD	0.467 (0.349–0.583)	0.778 (0.552–0.975)	742
	2003–2004	*	0.140 (<LOD–0.194)	0.304 (0.264–0.356)	0.629 (0.461–0.815)	0.970 (0.719–1.50)	648
	2007–2008	*	<LOD	0.509 (0.457–0.596)	0.966 (0.875–1.07)	1.33 (1.12–1.75)	573
	2009–2010	0.215 (0.192–0.240)	0.195 (0.180–0.218)	0.344 (0.314–0.400)	0.628 (0.489–0.822)	1.06 (0.714–1.38)	504
Non-Hispanic whites	1999–2000	*	<LOD	<LOD	<LOD	<LOD	589
	2001–2002	*	<LOD	0.412 (<LOD–0.455)	0.769 (0.667–0.894)	1.25 (1.05–1.40)	1,200
	2003–2004	0.263 (0.213–0.326)	0.269 (0.226–0.318)	0.539 (0.434–0.665)	1.13 (0.941–1.46)	2.34 (1.54–2.73)	1,075
	2007–2008	*	<LOD	0.780 (0.737–0.871)	1.36 (1.17–1.55)	2.00 (1.60–2.49)	1,083
	2009–2010	0.357 (0.308–0.414)	0.328 (0.288–0.384)	0.547 (0.485–0.644)	1.10 (0.897–1.40)	1.79 (1.35–3.02)	1,200

CI = confidence interval

Source: CDC 2015

2015). Urinary levels have remained steady over the temporal period for the age and gender groups shown in the tables and represent a broad mix of the general public.

In a study of pesticide residues collected from 1,000 adults, ranging in age from 20 to 59 years, living in the United States, 2,4-D was detected in 12% of samples at a mean concentration of <1 µg/L (Hill et al. 1995). The 95th percentile and maximum concentrations were reported as 1.8 and 37 µg/L, respectively.

In the Children's Total Exposure to Persistent Pesticides and Other Persistent Organic Pollutants (CTEPP) study, the exposures of 135 preschool children and their adult caregivers to 2,4-D at their homes in North Carolina and Ohio were examined in 2000 and 2001 (Morgan et al. 2008). Monitoring was performed over a 48-hour period, and personal (hand wipes and food) and environmental (air, soil, and dust) samples were collected. 2,4-D was detected in all types of environmental samples, with the highest frequency in carpet dust samples at 83% (median concentration of 47.5 ng/g) and 98% (median concentration of 156 ng/g) in North Carolina and Ohio, respectively. Detection frequencies in North Carolina and Ohio were 38 and 49% (maximum concentrations of 3.7 and 2.0 ng/m^3) for indoor air, 19 and 34% (maximum concentrations of 1.7 and 3.2 ng/m^3) for outdoor air, and 17 and 45% (maximum concentrations of 30.5 and 13.3 ng/g) for soil, respectively. Maximum concentrations of 2,4-D in personal exposure samples for adults in North Carolina and Ohio were 0.02 and 0.1 ng/cm^2 for hand wipes and 4.0 and 3.7 ng/g for solid food, respectively. 2,4-D was detected in >85% of the total samples collected. The median 2,4-D urinary concentrations in adults were 0.7 ng/mL for both North Carolina and Ohio residents. Morgan (2015) examined urinary levels of 2,4-D and other pesticide biomarkers and compared sociodemographic and lifestyle factors with exposure levels. Geometric mean urinary levels of 2,4-D (0.80 ng/mL [µg/L]) in urine of younger adults aged 20–35 years were significantly higher ($p=0.0025$) when compared to levels (0.54 ng/mL [µg/L]) in older adults aged 36–49 years. The study also indicated that sweet/salty snack consumption, time spent outside the home, and creatinine levels were significant ($p<0.05$) predictors of urinary 2,4-D levels.

Indoor air, outdoor air, and urine samples were analyzed for 2,4-D in a study assessing the exposure of 20 home gardeners and 19 bystanders living within the household using the product. (Harris et al. 1992). The homeowners were divided into groups that wore protective and non-protective clothing and applied both a granular and liquid formulation. The protective apparel group applying liquid 2,4-D reported no 2,4-D in air samples collected outside and only one detection at 6.0 µg/m^3 in indoor air. The protective group using granular 2,4-D reported no 2,4-D in indoor samples and three detections in outdoor air, with a mean concentration of 2.9 µg/m^3. No 2,4-D was detected in the urine of bystanders in either protective

group. Among the applicators, three had detections in urine at total concentrations of 108, 63, and 38 µg/person in 4 days, and these were all attributed to the applicator removing their gloves at some point during application. Analysis of urine samples collected from home gardeners 96 hours after application showed 2,4-D total body doses ranging from below detection to 0.0071 mg/kg of body weight. The total mean 2,4-D urine concentration of applicators using liquid and granular formulations were 203.6 and 18.8 µg/person in 4 days, respectively. Bystanders in both non-protective groups had no 2,4-D detections in urine. The highest exposures were found in the group wearing non-protective apparel and were associated with spills of the liquid formulation and dermal contact with the herbicide. There is a chance that bystanders could be exposed from treated turf grass immediately following application, although it has been shown that this may be <6% of the original amount of 2,4-D used.

Workers may be exposed to 2,4-D during mixing, loading, and applying, for both crop and non-agricultural uses (EPA 2005a). Families of workers may also be exposed to 2,4-D through home surfaces contaminated from contact with an applicator's hands or clothing. Deposition of 2,4-D contaminated dust or aerial dispersion from field spraying may also lead to surface contamination (Arbuckle et al. 2006).

In a biomonitoring study of exposure to 2,4-D in farm families with licensed applicators in Minnesota and South Carolina, 24-hour urine 2,4-D concentrations were collected 1 day before through 3 days after application (Alexander et al. 2007). For applicators (n=34), spouses (n=34), and children 4–17 years old (n=53), the median urine 2,4-D concentrations pre-application and 1 day after application were 2.1 and 73.1 µg/L, below the limit of detection and 1.2 µg/L, and 1.5 and 2.9 µg/L, respectively. At baseline, 2,4-D was detectable in the urine of 70% of the applicators, 41% of the spouses, and 62% of the children. The mean urine 2,4-D concentration in applicators and spouses the day before application, the day of application, 1 day after application, 2 days after application, and 3 days after application were 3.8 and 1.0, 29.1 and 1.0, 64.2 and 1.3, 45.3 and 1.4, and 28.3 and 1.3 µg/L, respectively. During and postapplication concentrations for applicators were substantially higher than baseline concentrations. Applicators who wore gloves to prevent direct skin contact had consistently lower urine 2,4-D concentrations, with the mean concentration for applicators not wearing gloves >7 times greater (236 compared to 44 µg/L). Exposure to spouses was determined to be primarily attributable to the level of contact with the application process, including their presence during mixing or application of 2,4-D. The geometric mean urinary levels of 2,4-D in 69 herbicide applicators were 7.8 and 25 µg/L prior to 2,4-D application and 1 day following application, respectively (Thomas et al. 2010a, 2010b). The mean absorbed dose estimated for 14 2,4-D broadcast and spray applications was 0.0027±0.0044 mg/kg/day. The mean

absorbed dose accounts from exposures from all sources, including application (dermal and inhalation) plus dietary ingestion and contact with 2,4-D containing surfaces in the home or farm.

In a study of repeated pesticide exposure to migrant and seasonal farmworkers in North Carolina, urine samples were collected from 196 farmworkers four times at monthly intervals in 2007 (Arcury et al. 2010). 2,4-D had at least one detection in 98% of farmworkers, and 86.7% had multiple detections.

While direct contact with 2,4-D during mixing, loading, application, or cleaning is the primary route of exposure for individuals living on a farm, indirect sources may also contribute. This includes contact with contaminated surfaces within the home (Arbuckle et al. 2006). In a biomonitoring study performed May through July 1996 to identify potential sources of 2,4 D exposure for families on farms, residues in drinking water and surface swipes of commonly touched surfaces with 32 Ontario farm homes were measured and compared to urinary concentrations found in applicators, spouses, and children. Surfaces tested were exterior door handles, refrigerator handles, kitchen faucet, washing machine knobs, bathroom faucet, wash-up faucet, telephone, toilet handle, and tractor steering wheel. 2,4-D was detected on all measured surfaces, with the highest levels found on the washing machine knob, wash-up faucet, and tractor steering wheel. For urine samples collected before application of 2,4-D, 66% of applicators, 44% of spouses, and 46% of children had a concentration ≥1 µg/L of 2,4 D, suggesting that 2,4-D used in previous seasons may be tracked indoors and persist on home surfaces. Mean concentrations of drinking water suggested that this is not an important route of exposure, as only 1% of homes had detectable levels of 2,4-D (Arbuckle et al. 2006).

A study was conducted measuring the levels of pesticides in urine and hand wipes among 24 farmer and 23 non-farmers in Iowa in the spring and summer of 2001 (Curwin et al. 2005a). Urine and hand wipe samples were collected from each person on two occasions, approximately 1 month apart. 2,4-D urinary concentrations were significantly higher in farmers who applied 2,4-D (mean of 13 µg/L), compared to farmers who had it commercially applied (mean of 1.6 µg/L), farmers who did not apply it (mean of 0.48 µg/L), and non-farmers (mean of 0.29 µg/L). It was shown that 2,4-D urine levels may be associated with time since application, amount of 2,4-D applied, and number of acres to which it was applied. None of the 21 hand wipe samples collected had detectable 2,4-D residues. Urinary levels of 2,4-D were measured in corn farmers from Iowa over the period of March to November 2002 and 2003 (Bakke et al. 2009). Statistically significant increases in 2,4-D levels were observed during the planting season as compared to pre-planting and the offseason; however, differences remained significant even after the

exclusion of urine samples obtained within 7 days of application, suggesting that exposure can continue well after application.

Curwin et al. (2005b) conducted a study of agricultural pesticide contamination in 25 farm homes and 25 nonfarm homes in Iowa by collecting air, surface wipe, and dust samples between May and August of 2001. Samples from 11 homes (5 farm homes and 6 nonfarm homes) were taken for 2,4-D detection. 2,4-D was found in 100% of farm and nonfarm dust samples, with concentrations of 0.0041–1.9 and 0.00099–5.3 ng/cm^2, respectively. In farm and nonfarm homes, 2,4-D adjusted mean concentrations in dust were highest in the entryway, 850 and 740 ng/g, respectively, while in the child's bedroom, the mean concentrations were 660 and 450 ng/g, respectively. All outdoor air (n=98) and indoor air samples (n=99) were below the limit of detection. Of the 82 house wipe and 48 vehicle wipe samples, 2,4-D was below the detection limit for all samples. This study is another example that agriculturally used 2,4-D may be an important source of home contamination.

In workers spraying 2,4-D in wheat fields, concentrations detected in 165 urine samples from 34 workers ranged from 35 to 400 µg/L (Aprea et al. 1997).

A summary of urinary concentrations 2,4-D in workers is presented in Table 6-4.

The National Occupational Exposure Survey (NOES) conducted by NIOSH in 1983 estimated that 471 workers employed at 94 facilities were potentially exposed to 2,4-D in the United States (RTECS 2009). The NOES database does not contain information on the frequency, concentration, or duration of exposure; the survey provides only estimates of workers potentially exposed to chemicals in the workplace.

6.6 EXPOSURES OF CHILDREN

This section focuses on exposures from conception to maturity at 18 years in humans. Differences from adults in susceptibility to hazardous substances are discussed in Section 3.7, Children's Susceptibility.

Children are not small adults. A child's exposure may differ from an adult's exposure in many ways. Children drink more fluids, eat more food, breathe more air per kilogram of body weight, and have a larger skin surface in proportion to their body volume than adults. A child's diet often differs from that of adults. The developing human's source of nutrition changes with age: from placental nourishment to

Table 6-4. Measured 2,4-D Urine Concentrations for Workers

Occupation	Number of samples	Geometric mean (µg/L)	Notes	Reference
Farmer (applicator)	34	3.8, 29.1, 64.2, 45.3, and 28.3	Day before, day of, 1 day after, 2 days after, and 3 days after application, respectively	Alexander et al. 2007
Herbicide applicator	69	7.8 and 25	Prior to and 1 day after application, respectively	Thomas et al. 2010a, 2010b
Farmer (applicator)	48	13		Curwin et al. 2005a
Sprayers in wheat fields	165	35–400 (range)	34 workers sampled	Aprea et al. 1997

breast milk or formula to the diet of older children who eat more of certain types of foods than adults. A child's behavior and lifestyle also influence exposure. Children crawl on the floor, put things in their mouths, sometimes eat inappropriate things (such as dirt or paint chips), and may spend more time outdoors. Children also are generally closer to the ground and have not yet developed the adult capacity to judge and take actions to avoid hazards (NRC 1993).

Children may be exposed to 2,4-D during and after its use in residential and recreational areas, such as on lawns or park grasses. Children may also be exposed when swimming in bodies of water that have been treated with 2,4-D (EPA 2005a). Children who live with farmworkers may also be exposed to 2,4-D from the clothing, boots, or containers brought into the home by household residents after a workday and spray drift proximal to fields, forests, and orchards (Arcury et al. 2007).

In a biomonitoring study of exposure to 2,4-D in farm families with licensed applicators in Minnesota and South Carolina, 24-hour urine 2,4-D concentrations were collected 1 day before through 3 days after application (Alexander et al. 2007). For children 4–17 years old (n=53), the median urine 2,4-D concentrations pre-application and 1 day after application were 1.5 and 2.9 µg/L, respectively. At baseline, 2,4-D was detectable in the urine of 62% of the children. The mean urine 2,4-D concentration in children the day before application, the day of application, 1 day after application, 2 days after application, and 3 days after application were 1.4, 2.1, 3.6, 3.5, and 3.4 µg/L, respectively. Younger children, 4–11 years old, had higher median post-application urine 2,4-D concentrations than older children, 12–17 years old (6.5 compared to 1.9 µg/L). Exposure to children was determined to be primarily attributable to the level of contact with the application process, including their presence during mixing or application of 2,4-D. Another study was performed to measure the level of pesticide urinary metabolites in 60 farmworker children 1–6 years old in North Carolina from July through August 2004 (Arcury et al. 2007). 2,4-D was detected in 41.7% of the 60 urine samples collected, with a median concentration of 0.23 µg/g creatinine.

Nishioka et al. (2001) performed a study to determine exposure to 2,4-D to young children (ages 5–14 years) in air and on surfaces (floors, table tops, and window sills) inside single-story Midwestern residences both before and after lawn application. 2,4-D was detected in indoor air and on all surfaces after application. It was determined that the main transport routes of 2,4-D into the home were from the homeowner applicator and by an active dog. No 2,4-D was detected in indoor air samples before application. The maximum indoor air concentrations during and after application were 17.7 and 10.8 ng/m^3, respectively. Post application, floor dust was concluded to be the major source of 2,4-D in

the air, on tables, and on window sills by resuspension. Postapplication floor dust concentrations ranged from approximately 1 to 200 μg/m^2, compared to 0.2–1.0 μg/m^2 for dust levels prior to application. The concentrations of 2,4-D measured in occupied homes postapplication on carpets, bare floors, table tops, and window sills were <0.1–228, <0.01–23, 0.3–27, and 0.5–22 μg/m^2, respectively. It was estimated that dietary ingestion was the main source of exposure for young children before lawn application of 2,4-D, but during postapplication periods, dietary ingestion (53%), nondietary ingestion (41%), and dermal penetration (4%) were the main pathways. Postapplication exposure levels from nondietary ingestion by contact with floors and contact with table tops were estimated to be 1–10 and 0.2–30 μg/day, respectively, which are estimated to be about 10 times higher than levels before application. Dust samples collected from the homes of 513 subjects residing in Detroit, Michigan, the state of Iowa, Los Angeles, California, and Seattle, Washington had an arithmetic mean and geometric mean concentration of 2,422 and 419 ng/g, respectively (Colt et al. 2004). Seventy-eight percent of all of the samples tested were positive for 2,4-D. Samples collected in Iowa had the greatest geometric mean concentration of 2,4-D (1,512 ng/g), followed by Detroit (606 ng/g), Seattle (374 ng/g), and Los Angeles (87 ng/g).

The National Health and Nutrition Examination Survey (NHANES) uses biomonitoring to provide estimates of exposure to the civilian U.S. population. Chemicals and their metabolites are measured in subsets of participants aged 6–59 years old, meant to be a representative sample of the population. Urinary levels of 2,4-D in children 6–11 and 12–19 years old were measured in NHANES samples assessing exposure from years 1999–2010 (CDC 2015). For survey years 1999–2000 and 2001–2002, no geometric mean urinary concentration of the 2,4-D could be calculated because the proportion of results below the detection limit was too high to provide a valid result. These results are summarized in Tables 6-2 and 6-3 (CDC 2013). The results suggest that urinary levels of 2,4-D in children have remained relatively unchanged over the temporal period, but slightly higher levels have been observed in children as compared to adults.

In the CTEPP study, the exposures of 135 preschool children and their adult caregivers to 2,4-D at their homes in North Carolina and Ohio were examined in 2000 and 2001 (Morgan et al. 2008). Monitoring was performed over a 48-hour period, and personal (hand wipes and food) and environmental (air, soil, and dust) samples were collected. 2,4-D was detected in all types of environmental samples, with the highest frequency in carpet dust samples at 83% (median concentration of 47.5 ng/g) and 98% (median concentration of 156 ng/g) in North Carolina and Ohio, respectively. Detection frequency in North Carolina and Ohio were 38 and 49% (maximum concentrations of 3.7 and 2.0 ng/m^3) for indoor air, 19 and 34% (maximum concentrations of 1.7 and 3.2 ng/m^3) for outdoor air, and 17 and 45% (maximum

concentrations of 30.5 and 13.3 ng/g) for soil, respectively. Maximum concentrations of 2,4-D in personal exposure samples for children in North Carolina and Ohio were 0.04 and 0.1 ng/cm^2 for hand wipes and 4.4 and 20.2 ng/g for solid food, respectively. 2,4-D was detected in >85% of the total samples collected. The median 2,4-D urinary concentrations in children were 0.5 and 1.2 ng/mL in North Carolina and Ohio, respectively. Morgan et al. (2014) estimated the potential intakes of 2,4-D from different routes using data from 129 preschool children from North Carolina in the CTEPP study. The daily intake dose was calculated as 4.981 ng/kg/day, with the largest intake arising from dietary exposure (4.84 ng/kg/day).

In a study of urine collected from 197 children in Arkansas, 20% had detectable levels of 2,4-D, and the 95th percentile and maximum concentrations were reported as 3 and 9 µg/L, respectively (Hill et al. 1989).

6.7 POPULATIONS WITH POTENTIALLY HIGH EXPOSURES

As discussed in Section 6.5, occupational exposure to workers during mixing, loading, and application of 2,4-D will likely result in higher-than-average exposures to this substance (EPA 2005a). The EPA RED outlines the Personal Protective Equipment (PPE) requirements for 2,4-D labeling for liquids, wettable powders, and water dispersible granules as well as pure granular formulations (EPA 2005a). In general, in order to reduce exposure, mixers, loaders, applicators flaggers, and other handlers should wear long-sleeved shits/pants, shoes, and socks and chemical resistant gloves. Homeowners and their families who use 2,4-D for lawn treatment also have a higher potential for exposure than people who do not apply 2,4-D to their lawns. Homeowners applying 2,4-D should follow similar labeling procedures to reduce exposure. Families of workers may also be exposed through home surfaces contaminated from contact with an applicator's hands or clothing. In addition, families living proximal to treated fields, orchards, and managed forests/timber may have greater exposure than the general population.

Comparing urinary 2,4-D levels from the NHANES, 1999–2010, report to data from occupationally exposed workers indicates that urinary 2,4-D levels can be up to 100 times greater for workers shortly after application as compared to the general population in the 50th percentile (Alexander et al. 2007; CDC 2015; Thomas et al. 2010a, 2010b).

6.8 ADEQUACY OF THE DATABASE

Section 104(i)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether

adequate information on the health effects of 2,4-D is available. Where adequate information is not available, ATSDR, in conjunction with NTP, is required to assure the initiation of a program of research designed to determine the health effects (and techniques for developing methods to determine such health effects) of 2,4-D.

The following categories of possible data needs have been identified by a joint team of scientists from ATSDR, NTP, and EPA. They are defined as substance-specific informational needs that if met would reduce the uncertainties of human health assessment. This definition should not be interpreted to mean that all data needs discussed in this section must be filled. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

6.8.1 Identification of Data Needs

Physical and Chemical Properties. The physical-chemical properties of 2,4-D are provided in Chapter 4. Important properties such as melting point, boiling point, vapor pressure, solubility, log K_{ow} and Henry's Law constant are available. No data needs are identified.

Production, Import/Export, Use, Release, and Disposal. According to the Emergency Planning and Community Right-to-Know Act of 1986, 42 U.S.C. Section 11023, industries are required to submit substance release and off-site transfer information to the EPA. The TRI, which contains this information for 2013, became available in February 2015. This database is updated yearly and should provide a list of industrial production facilities and emissions.

Environmental Fate. The environmental fate and transport of 2,4-D is understood and no data needs are identified. The mobility of 2,4-D in soils is expected to be high based on measured K_{oc} values; however, detection of 2,4-D in groundwater is infrequent since it degrades rapidly in soil. Volatilization is generally considered low. Hydrolysis in acidic soils and photolysis may result in some degradation of 2,4-D. Biodegradation primarily accounts for the removal of 2,4-D from the environment.

Bioavailability from Environmental Media. 2,4-D has been detected in aquatic and terrestrial organisms (Schultz and Whitney 1974) and is therefore bioavailable to some extent from environmental media; however, elimination from the organisms was rapid. Aerobic biodegradation reduces its bioavailability. No data needs are identified.

Food Chain Bioaccumulation. Measured BCFs of 2,4-D in fish suggest that bioaccumulation in aquatic organisms is not high. No data needs are identified.

Exposure Levels in Environmental Media. Reliable monitoring data for the levels of 2,4-D in contaminated media at hazardous waste sites are needed so that the information obtained on levels of 2,4-D in the environment can be used in combination with the known body burden of 2,4-D to assess the potential risk of adverse health effects in populations living in the vicinity of hazardous waste sites.

Exposure Levels in Humans. Humans are exposed to 2,4-D mainly by dermal exposure during application as an herbicide. Populations may also be exposed by transport of 2,4-D into residential homes from agricultural spray drift, volatilization, soil or dust resuspension, track-in on shoes, and on clothing. Adequate biomonitoring data are available to assess 2,4-D exposure to the general population of the United States. Continued monitoring of the general U.S. population through the NHANES program can provide information on the trend of exposure to 2,4-D and identify subsets in the population with the highest levels of exposure.

Exposures of Children. Children are exposed to 2,4-D mainly by dermal exposure to residue transported into homes from applicators and from direct contact with treated residential lawns. Adequate biomonitoring data are available to assess 2,4-D exposure to children of the United States. Continued monitoring through the NHANES program is needed in order to understand future exposures. Additional research on exposures of neonates and young children of workers who handle 2,4-D is needed and justifiable. No human data were located regarding 2,4-D in breast milk and this is a data need.

Child health data needs relating to susceptibility are discussed in Section 3.12.2, Identification of Data Needs: Children's Susceptibility.

Exposure Registries. No exposure registries for 2,4-D were located. This substance is not currently one of the compounds for which a sub-registry has been established in the National Exposure Registry. The substance will be considered in the future when chemical selection is made for sub-registries to be established. The information that is amassed in the National Exposure Registry facilitates the epidemiological research needed to assess adverse health outcomes that may be related to exposure to this substance.

6.8.2 Ongoing Studies

As part of the Fourth National Health and Nutrition Evaluation Survey, the Environmental Health Laboratory Sciences Division of the National Center for Environmental Health, Centers for Disease Control and Prevention, will be analyzing urine samples for 2,4-D. These data will give an indication of the frequency of occurrence and background levels of this compound in the general population.

No ongoing environmental fate studies for 2,4-D were identified using the NIH RePORTER (2015) or the Defense Technical Information Center (DTIC) online database.

7. ANALYTICAL METHODS

The purpose of this chapter is to describe the analytical methods that are available for detecting, measuring, and/or monitoring 2,4-D, its metabolites, and other biomarkers of exposure and effect to 2,4-D. The intent is not to provide an exhaustive list of analytical methods. Rather, the intention is to identify well-established methods that are used as the standard methods of analysis. Many of the analytical methods used for environmental samples are the methods approved by federal agencies and organizations such as EPA and the National Institute for Occupational Safety and Health (NIOSH). Other methods presented in this chapter are those that are approved by groups such as the Association of Official Analytical Chemists (AOAC) and the American Public Health Association (APHA). Additionally, analytical methods are included that modify previously used methods to obtain lower detection limits and/or to improve accuracy and precision.

7.1 BIOLOGICAL MATERIALS

Table 7-1 lists the methods used for determining 2,4-D in biological samples. The principal separation and detection methods of 2,4-D in biological samples include gas chromatography (GC) or high-performance liquid chromatography (HPLC), in conjunction with diode-array detection (DAD) or electron capture detection (ECD). GC requires 2,4-D derivatization to a more stable compound for analysis. The most common derivatization agents for alkyl esters, including 2,4-D, in urine consist of dimethylsulfate via methylation and other agents such as diazomethane, diazoethane, and pentafluorobenzylbromide (PFB-Br) (Aprea et al. 1997). HPLC does not require derivatization of 2,4-D, allowing for higher detection limits.

2,4-D is excreted in urine mostly as a free acid, with only a small percentage in conjugated form, and therefore, levels of free acid 2,4-D can be used as indicators of exposure to the parent compound and its salts (Aprea et al. 1997). Several methods have been described for the detection of 2,4-D in urine. Aprea et al. (1997) describe two separate methods. The first method uses HPLC followed by DAD and has a detection limit of 15 µg/L. The second method uses PFB-Br derivatization for analysis by GC followed by ECD detection. The detection limit for this method is 1 µg/L. Both methods employ dichloromethane as an extraction solvent.

Table 7-1. Analytical Methods for Determining 2,4-D in Biological Samples

Sample matrix[a]	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Human urine	Hydrolysis using KOH. Liquid-liquid extraction into methylene chloride. Derivatization with diazomethane.	GC/MSD	5.0 ppb	90.3% (14.31% RSD)	Hughes et al. 2001
Human urine	Hydrolysis with mineral acid. Extraction by acid/base partitioning. Derivatization with ethereal diazomethane.	GC/ECD	0.05 mg/L	97%	Draper 1982
Human urine	Extraction with 12 mL dichloromethane. Organic extract dehydration with anhydrous sodium sulfate, followed by evaporation. Silica SPE purification.	HPLC/DAD	15 µg/L	81% (6.2 CV at 125.0 µg/L)	Aprea et al. 1997
Human urine	Extraction with 12 mL dichloromethane. Organic extract dehydration with anhydrous sodium sulfate, followed by evaporation. Derivatization with 200 µL solution of PFB-Br in acetone (1:100), 15 µL of 60% aqueous solution of potassium carbonate, and 4mL acetone. Silica SPE purification.	GC/ECD	1 µg/L	87% (8% CV at 30.0 µg/L)	Aprea et al. 1997
Human urine	Addition of 10 mL 0.1M acetate buffer (pH 4.5) solution. Addition of 3N NaOH for pH adjustment. Extraction with 7.5 mL diethyl ether-methylene chloride (4:1).	HPLC/MS/MS	0.29 µg/L	92% (7.1 % RSD at 5 µg/L)	Baker et al. 2000
Human urine	Homogenization. Enzymatic hydrolysis. Addition of 10mL acetonitrile. Dissolution in methanol:water (10:90).	LC/HRMS	0.8 ng/mL	98% (17% RSD)	Roca et al. 2014
Animal kidney tissue	Addition of 5 g sand and 25 g sodium sulfate. Ground to a powder. Soxhlet extraction with diethyl ether. Clean up by anion exchange SPE.	LC/MS/MS	0.02 mg/kg	85% (19% RSD at 1 mg/kg)	Charlton et al. 2009

Table 7-1. Analytical Methods for Determining 2,4-D in Biological Samples

Sample matrix[a]	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Animal tissue	Homogenization with ethanol. Soxhlet extraction with ethanol. Clean up with NaOH. Hydrolyzed to 2,4-dichlorophenol. Clean up with steam distillation.	GC	0.05 ppm	Muscle: 84% Liver: 91% Kidney: 68% Renal fat: 92% Body fat: 90%	Clark et al. 1967

[a]2,4-D is the target analyte unless otherwise specified.

CV = coefficient of variation; DAD = diode-array detector; ECD = electron capture detector; GC = gas chromatography; HPLC = high-performance liquid chromatography; HRMS = high resolution mass spectrometry; KOH = potassium hydroxide; LC = liquid chromatography; MSD = mass selective detection; MS/MS = tandem mass spectrometry; NaOH = sodium hydroxide; PFB-Br = pentafluorobenzylbromide; RSD = relative standard deviation; SPE = solid-phase extraction.

Another method for detecting 2,4-D in urine uses HPLC followed by tandem mass spectrometry (MS/MS). The extraction solvent used in this method was diethyl ether-methylene chloride, and the detection limit for this method is 0.29 µg/L (Baker et al. 2000). A method for detecting pesticides in urine using liquid chromatography followed by high resolution MS was described, with a reported 2,4-D detection limit of 0.8 ng/mL (Roca et al. 2014).

Dermal exposure to 2,4-D by applicators can be assessed by using dermal exposure pads or hand rinse procedures followed by an appropriate analytical method. Sell and Maitlen (1983) describe a method using absorbent gauze swipe pads attached to clothing to capture 2,4-D residues followed by extraction with methanol and analysis by GC.

Draper (1982) describes a multi-residue procedure for the determination of acidic herbicide residues in human urine. This method employs derivatization with ethereal diazomethane and electron capture GC detection. The detection limit was reported as 0.05 mg/L. Another method using derivatization with diazomethane followed by GC and mass selective detection was described for analysis of 2,4-D in human urine (Hughes et al. 2001). The reported limit of detection was 5.0 ppb.

Charlton et al. (2009) described a method for the detection of 2,4-D in animal kidney tissue that uses liquid chromatography with electrospray MS/MS. Samples are first extracted with diethyl ether followed by anion exchange solid phase extraction. The limit of detection for this method is reported as 0.02 mg/kg. Another method using GC detection is described for animal tissue and has a detection limit of 0.05 ppm (Clark et al. 1967).

7.2 ENVIRONMENTAL SAMPLES

Table 7-2 lists the methods used for determining 2,4-D in environmental samples. Most methods involve the esterification of 2,4-D to its methyl ester for detection. The principal separation and detection methods of 2,4-D and degradation products in environmental samples include GC or HPLC, in conjunction with ultraviolet (UV) or ECD.

Three NIOSH methods (Methods 5602, 9200, and 9201) have been used to analyze 2,4-D in occupational air, hand wash, and dermal patch samples (NIOSH 1998a, 1998b, 1998c). These methods employ the use of HPLC with ECD. The derivatization agent employed in these methods is diazomethane. Detection limits are as low as 0.0005 µg/mL.

Table 7-2. Analytical Methods for Determining 2,4-D in Environmental Samples

Sample matrix[a]	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Air	Air is drawn through a cassette with a glass fiber filter. Desorption with methanol.	HPLC/UV (284 nm) (NIOSH Method 5001)	0.015 mg/m^3	97% (5–20 µg/m^3, 100 L sample)	NIOSH 1994
Air	Air is drawn through a glass tube with a quartz fiber filter and XAD-2 adsorbent. Extraction with 2 mL 10% methanol/90% MTBE with diazomethane derivatizing agent.	GC/ECD (NIOSH Method 5602)	0.03 µg/sample	91%	NIOSH 1998a
Occupational air	Hand wash. Insert hand into a bag containing 150 mL of isopropanol. Add 0.5 mL of diazomethane derivatizing agent.	GC/ECD (NIOSH Method 9200)	0.001 µg/mL	101.9%	NIOSH 1998b
Occupational air	Dermal patch. Polyurethane foam pad attached to clothing or skin. Desorption of sample using 40 mL isopropanol with diazomethane derivatizing agent.	GC/ECD (NIOSH Method 9201)	0.0025 µg/mL	75% (day 1) 97.3% (day 30)	NIOSH 1998c
Water	Add sample to a test tube containing polyclonal antibodies. Incubate for 10 minutes. Add 2,4-D enzyme conjugate. Wash and add clear substrate. Incubate for 10 minutes and add stop solution (diluted sulfuric acid).	Immunoassay/ photometer (Abraxis Tube Kit)	2 ppb	No data	Abraxis 2007
Water	Add sample to plate kit. Add 2,4-D HRP conjugate and incubate for 60 minutes. Wash and add clear substrate. Incubate for 30 minutes and add stop solution (diluted hydrochloric acid).	Immunoassay/ photometer (Abraxis Plate Kit)	2 ppb	No data	Abraxis 2008
Water	Sample converted to sodium salt with NaOH. Extraction with ethyl ether and conversion to methyl ester with diazomethane.	GC/ECD (Method ASTM D5317)	0.2 µg/L	92% (13.1% RSD)	NEMI 2011

Table 7-2. Analytical Methods for Determining 2,4-D in Environmental Samples

Sample matrix[a]	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Water	Water is pumped through an SPE cartridge with a graphitized-carbon filter. Wash using 6 mL of 80:20 (v/v) methylene chloride and methanol. Elution using 8 mL of 80:20 (v/v) methylene chloride and methanol with TFAA at 0.2%.	HPLC/PDA-UV (Method O-1131-95)	0.013 µg/L	50% (9% RSD)	USGS 1996
Water	Water is pumped through an SPE cartridge with a graphitized-carbon filter. Two elutions with 1.5 mL methanol, followed by 13 mL of 80:20 (v/v) methylene chloride and methanol with TFAA at 0.2%.	RP-HPLC/MS (Method O-2060-01)	0.0109 µg/L	96% (14% RSD)	USGS 2001
Groundwater and drinking water	Hydrolysis to chlorinated esters using 6N NaOH. Solvent wash with methylene chloride. Acidification with H_3PO_4. Extraction with ethyl ether. Converted to methyl ester with diazomethane.	GC/ECD (Method 515.1)	0.078 µg/L	90% (14% RSD)	EPA 1995a
Groundwater and drinking water	Hydrolysis to chlorinated esters using 6N NaOH. Solvent wash with methylene chloride. Acidification with H_3PO_4. Extraction with resin based extraction disk. Converted to methyl ester with diazomethane.	GC/ECD (Method 515.2)	0.28 µg/L	96% (38% RSD)	EPA 1995b
Groundwater, drinking water, raw source water, waste water	Hydrolysis to chlorinated esters using 4N NaOH. Acidification with H_3PO_4. Extraction with 4 mL MTBE. Converted to methyl ester with diazomethane.	GC/ECD (Method 515.3)	0.35 µg/L	137% (6.4% RSD)	EPA 1996a

Table 7-2. Analytical Methods for Determining 2,4-D in Environmental Samples

Sample matrix[a]	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Ground water and surface water	Hydrolysis to chlorinated esters using 4N NaOH. Wash with hexane:MTBE (90:10, v/v) mixture. Acidification with H_3PO_4. Extraction with 4 mL MTBE. Converted to methyl ester with diazomethane.	GC/ECD (Method 515.4)	0.55 µg/L (40 mL sample)	98% (5.2% RSD)	EPA 2000
Groundwater and drinking water	Hydrolysis to chlorinated esters using 6N NaOH. Acidification with H_3PO_4, filtered, and extracted.	HPLC/PDA-UV (Method 555)	1.3 µg/L (20 mL sample)	112% (4.2% RSD)	NEMI 1992
Leachates and liquid wastes	Sample filtration; pH adjustment if needed.	RP-HPLC/UV (235 nm)	0.59 mg/L	91.1% (7.7% RSD) at 5 µg/mL; 106.8% (3.2% RSD) at 20 µg/mL	DOE 1997
Water, soil, waste	Water samples extracted with diethyl ether and then esterified with diazomethane. Soil and waste samples extracted and esterified with diazomethane.	capillary GC/ECD (Method 8151A)	0.2 µg/L (aqueous sample) 0.11 µg/kg (soil sample)	131% (27.5% RSD) (aqueous sample) 84.3% (5.3% RSD) (soil sample)	EPA 1996b
Water, aqueous suspended sediment	Extraction with diethyl ether or MTBE from acidified water sample. Hydrolysis with potassium hydroxide. Esterification to methyl ester using boron trifluoride-methanol.	GC/ECD (Method O-1105)	0.01 µg/L	75% (10% RSD)	USGS 1987

ASTM = American Society for Testing and Materials; ECD = Ni electron capture detector; EPA = Environmental Protection Agency; GC = gas chromatography; HPLC = high-performance liquid chromatography; H_3PO_4 = phosphoric acid; MS = mass spectrometry; MTBE = methyl t-butyl ether; NaOH = sodium hydroxide; NEMI = National Environmental Methods Index; NIOSH = National Institute for Occupational Safety and Health; PDA = photodiode array; RP = reverse phase; RSD = relative standard deviation; SPE = solid phase extraction; TFAA = trifluoroacetic acid; USGS = U.S. Geological Survey; UV = ultraviolet absorbance detection.

Two methods have also been described that use immunoassay kits for the detection of 2,4-D in water. In these kits, 2,4-D competes with an enzyme conjugate for binding sites to 2,4-D antibodies. After incubation and washing, a clear substrate is added that causes a bound enzyme conjugate to turn a blue color. After another incubation period, the reaction is stopped using a solution containing diluted sulfuric or hydrochloric acid and the color of the samples is analyzed using a photometer. The detection limit for these methods is 2 ppb (Abraxis 2007, 2008).

The American Society for Testing and Materials (ASTM) Method D5317 uses a diazomethane derivatizing agent, similar to the NIOSH methods for air analysis, to convert 2,4-D into its methyl ester for determination of the compound in water (Abraxis 2007, 2008). The detection limit for this method is 0.2 µg/L.

Several methods have been described by the EPA's National Exposure Research Laboratory (NERL) for the detection of chlorinated acids, including 2,4-D, in groundwater, drinking water, raw source water, and/or waste waters. Methods 515.1 and 515.2 determine the concentration of 2,4-D using solvent and liquid-solid extraction, respectively, and analysis via GC and ECD, with detection limits of 0.078 and 0.28 µg/L, respectively (EPA 1995a, 1995b). Methods 515.3 and 515.4 use liquid-liquid extraction and GC/ECD analysis, with detection limits of 0.35 and 0.055 µg/L, respectively (EPA 1996a, 2000). Method 555 uses HPLC with a photodiode array UV detector and the detection limit for 2,4-D is 1.3 µg/L (NEMI 1992). The U.S. Geological Survey's (USGS) National Water Quality Laboratory (NWQL) also describes a method (O-1131-95) using solid-phase extraction (SPE) and HPLC with a photodiode array UV detector to determine pesticides, including 2,4-D in water. The reported detection limit for 2,4-D is 0.013 µg/L (USGS 1996).

The USGS NQWL describes a procedure (Method O-2060-01) for the determination of pesticides, including 2,4-D, in water that involves SPE and HPLC coupled with MS and has a reported detection limit of 0.0109 mg/L (USGS 2001).

A method for determining acidic semi-volatile compounds, including 2,4-D, in leachates and aqueous liquid-waste samples has been described. The method is a direct analysis using reverse-phase HPLC and UV absorbance detection, so other than filtration, no sample preparation is required. The detection limit for 2,4-D is 0.59 mg/L (DOE 1997).

The USGS NQWL described a method (O-1105) to determine chlorophenoxy acids, such as 2,4-D, in water and water-suspended sediments. The procedure involves esterification with boron trifluoride-methanol followed by separation and detection using GC and ECD. The detection limit was reported as 0.01 µg/L (USGS 1987)

EPA's Office of Solid Waste (OSW) describes a method for determining chlorinated herbicides, including 2,4-D, in water, soil, and waste samples. Samples are extracted and derivatized with diazomethane or pentafluorobenzyl bromide. Analysis is done by capillary GC with ECD, reporting 2,4-D detection limits of 0.2 µg/L for aqueous samples and 0.11 µg/kg for soil samples (EPA 1996b).

7.3 ADEQUACY OF THE DATABASE

Section 104(i)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of 2,4-D is available. Where adequate information is not available, ATSDR, in conjunction with NTP, is required to assure the initiation of a program of research designed to determine the health effects (and techniques for developing methods to determine such health effects) of 2,4-D.

The following categories of possible data needs have been identified by a joint team of scientists from ATSDR, NTP, and EPA. They are defined as substance-specific informational needs that if met would reduce the uncertainties of human health assessment. This definition should not be interpreted to mean that all data needs discussed in this section must be filled. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

7.3.1 Identification of Data Needs

Methods for Determining Biomarkers of Exposure and Effect. The available methods for the determination of 2,4-D in biological samples are adequate. However, to examine further possible exposures of human neonates and infants to 2,4-D and its relatives, precise and accurate methods for determination of these compounds at low levels in breast milk are needed. Various methods exist for determination of 2,4-D in urine (Aprea et al. 1997; Baker et al. 2000; Hughes et al. 2001; Roca et al. 2014) and tissue (Charlton et al. 2009; Clark et al. 1967). While there are methods for the determination of 2,4-D in blood, a detailed source was not be found in the literature. Methods for detection of 2,4-D in

water, air, soil, and waste samples are available (DOE 1997; EPA 1995a, 1995b; 1996b, 2000; NIOSH 1994, 1998a, 1998b, 1998c; USGS 1987, 1996, 2001).

Methods for Determining Parent Compounds and Degradation Products in Environmental Media. 2,4-D can be analyzed in water, air, soil, and waste samples with reasonable selectivity and sensitivity (DOE 1997; EPA 1995a, 1995b; 1996b, 2000; NIOSH 1994, 1998a, 1998b, 1998c; USGS 1987, 1996, 2001). Therefore, there is a reasonable database in this area.

7.3.2 Ongoing Studies

No ongoing studies for 2,4-D were identified using the NIH RePORTER (2015) or the DTIC online database.

8. REGULATIONS, ADVISORIES, AND GUIDELINES

MRLs are substance specific estimates that are intended to serve as screening levels. They are used by ATSDR health assessors and other responders to identify contaminants and potential health effects that may be of concern at hazardous waste sites.

The international and national regulations, advisories, and guidelines regarding 2,4-D in air, water, and other media are summarized in Table 8-1.

ATSDR has derived an intermediate-duration oral MRL of 0.009 mg/kg/day for 2,4-D based on a $BMDL_{RD05}$ of 0.93 mg 2,4-D/kg/day for reduced rat pup body weight on PND 16 following maternal exposure to 2.5 mg 2,4-D/kg/day on postpartum days 1–16 (Stürtz et al. 2010). An uncertainty factor of 100 was used (10 for animal to human extrapolation and 10 for human variability). The intermediate-duration oral MRL was also adopted as acute-duration oral MRL for 2,4-D.

EPA's Office of Pesticide Program's Registration Eligibility Decision (EPA 2005a) derived a reference dose (RfD) of 0.005 mg/kg/day for 2,4-D based on a NOAEL of 5 mg/kg/day for body weight effects as well as alterations in hematology and clinical parameters in Fischer rats in a 2-year unpublished study.

Regulations, advisories, and guidelines change. It is highly recommended that the public check for the most recent regulations, advisories, and guidelines with the agencies creating them to ensure that the public has up-to-date information.

Table 8-1. Regulations, Advisories, and Guidelines Applicable to 2,4-D

Agency	Description	Information	Reference
INTERNATIONAL			
Guidelines:			
IARC	Carcinogenicity classification	2B[a]	IARC 2016
WHO	Air quality guidelines	No data	WHO 2010
	Drinking water quality guidelines		WHO 2011
	Guideline value	0.03 mg/L[b]	
	ADI	0–0.01 mg/kg body weight[c]	
NATIONAL			
Regulations and guidelines:			
a. Air			
ACGIH	TLV (8-hour TWA)	10 mg/m^3 [d,e]	ACGIH 2015
AIHA	ERPGs	No data	AIHA 2014
DOE	PACs		DOE 2012a
	PAC-1[f]	14 mg/m^3	
	PAC-2[f]	14 mg/m^3	
	PAC-3[f]	500 mg/m^3	
EPA	AEGLs	No data	EPA 2015a
	Hazardous air pollutant	Yes[g]	EPA 2013a
NIOSH	REL (10-hour TWA)	10 mg/m^3	NIOSH 2015
	IDLH	100 mg/m^3	
OSHA	PEL (8-hour TWA) for general industry	10 mg/m^3	OSHA 2013 29 CFR 1910.1000, Table Z-1
	PEL (8-hour TWA) for shipyards	10 mg/m^3	OSHA 2014a 29 CFR 1915.1000 Table Z
	PEL (8-hour TWA) for construction	10 mg/m^3	OSHA 2014b 29 CFR 1926.55 Appendix A
b. Water			
EPA	Designated as hazardous substances in accordance with Section 311(b)(2)(A) of the Clean Water Act	Yes	EPA 2013b 40 CFR 116.4
	Drinking water standards and health advisories		EPA 2012
	1-day health advisory for a 10-kg child	1 mg/L	
	10-day health advisory for a 10-kg child	0.3 mg/L	
	DWEL	0.2 mg/L	

Table 8-1. Regulations, Advisories, and Guidelines Applicable to 2,4-D

Agency	Description	Information	Reference
NATIONAL (*cont.*)			
	National primary drinking water standards	No data	EPA 2009
	MCL	0.07 mg/L	
	Public Health Goal	0.07 mg/L	
	National recommended water quality criteria: human health for the consumption of		EPA 2015b
	Water plus organism	1300 µg/L	
	Organism only	12,000 µg/L	
	Reportable quantities of hazardous substances designated pursuant to Section 311 of the Clean Water Act	100 pounds	EPA 2013c 40 CFR 117.3
c. Food			
FDA	EAFUS	No data[h]	FDA 2013
	Allowable level in bottled water	0.07 mg/L	FDA 2014 21 CFR 165.110
d. Other			
ACGIH	Carcinogenicity classification	A4[i]	ACGIH 2015
EPA	Carcinogenicity classification	D[j]	EPA 2005a
	RfC	No data	
	OPP's RfD	5.0×10^{-3} mg/kg/day	EPA 2005a
	Superfund, emergency planning, and community right-to-know		EPA 2014a 40 CFR 302.4
	Designated CERCLA hazardous substance and reportable quantity	100 pounds	
	Effective date of toxic chemical release reporting	01/01/1987	EPA 2013d 40 CFR 372.65
	TSCA chemical lists and reporting periods	No data	EPA 2014b 40 CFR 712.30
DHHS	Carcinogenicity classification	No data	NTP 2014

[a]Group 2B: Possibly carcinogenic to humans.
[b]The guideline value applies to 2,4-D, as salts and esters of 2,4-D are rapidly hydrolyzed to the free acid in water. Levels in water usually occur below 0.5 µg/L, although concentrations as high as 0.3 mg/L have been measured.
[c]The ADI applies to the sum of 2,4-D and its salts and esters, expressed as 2,4-D, on the basis of a NOAEL of 1 mg/kg body weight per day in a 1-year study of toxicity in dogs (for a variety of effects, including histopathological lesions in kidneys and liver) and a 2-year study of toxicity and carcinogenicity in rats (for renal lesions).
[d]Skin notation: refers to the potential significant contribution to the overall exposure by the cutaneous route, including mucous membranes and the eyes, either by contact with vapors or, of probable greater significance, by direct skin contact with the substance.
[e]Inhalable fraction.
[f]Definitions of PAC terminology are available from U.S. Department of Energy (DOE 2012b).
[g]2,4-D, salts and esters.
[h]The EAFUS list of substances contains ingredients added directly to food that FDA has either approved as food additives or listed or affirmed as GRAS.
[i]A4: not classifiable as a human carcinogen.

Table 8-1. Regulations, Advisories, and Guidelines Applicable to 2,4-D

Agency	Description	Information	Reference

[j]Not classifiable as to human carcinogenicity.

2,4-D = 2,4-dichlorophenoxyacetic acid; ACGIH = American Conference of Governmental Industrial Hygienists; ADI = acceptable daily intake; AEGL = acute exposure guideline levels; AIHA = American Industrial Hygiene Association; CERCLA = Comprehensive Environmental Response, Compensation, and Liability Act; CFR = Code of Federal Regulations; DHHS = Department of Health and Human Services; DOE = Department of Energy; DWEL = drinking water equivalent level; EAFUS = Everything Added to Food in the United States; EPA = Environmental Protection Agency; ERPG = emergency response planning guidelines; FDA = Food and Drug Administration; GRAS = Generally Recognized As Safe; IARC = International Agency for Research on Cancer; IDLH = immediately dangerous to life or health; IRIS = Integrated Risk Information System; MCL = maximum contaminant level; NIOSH = National Institute for Occupational Safety and Health; NTP = National Toxicology Program; OPP = Office of Pesticide Programs; OSHA = Occupational Safety and Health Administration; PAC = Protective Action Criteria; PEL = permissible exposure limit; RCRA = Resource Conservation and Recovery Act; REL = recommended exposure limit; RfC = inhalation reference concentration; RfD = oral reference dose; TLV = threshold limit values; TSCA = Toxic Substances Control Act; TWA = time-weighted average; WHO = World Health Organization.

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10. GLOSSARY

Absorption—The taking up of liquids by solids, or of gases by solids or liquids.

Acute Exposure—Exposure to a chemical for a duration of 14 days or less, as specified in the Toxicological Profiles.

Adsorption—The adhesion in an extremely thin layer of molecules (as of gases, solutes, or liquids) to the surfaces of solid bodies or liquids with which they are in contact.

Adsorption Coefficient (K_{oc})—The ratio of the amount of a chemical adsorbed per unit weight of organic carbon in the soil or sediment to the concentration of the chemical in solution at equilibrium.

Adsorption Ratio (Kd)—The amount of a chemical adsorbed by sediment or soil (i.e., the solid phase) divided by the amount of chemical in the solution phase, which is in equilibrium with the solid phase, at a fixed solid/solution ratio. It is generally expressed in micrograms of chemical sorbed per gram of soil or sediment.

Benchmark Dose (BMD)—Usually defined as the lower confidence limit on the dose that produces a specified magnitude of changes in a specified adverse response. For example, a BMD_{10} would be the dose at the 95% lower confidence limit on a 10% response, and the benchmark response (BMR) would be 10%. The BMD is determined by modeling the dose response curve in the region of the dose response relationship where biologically observable data are feasible.

Benchmark Dose Model—A statistical dose-response model applied to either experimental toxicological or epidemiological data to calculate a BMD.

Bioconcentration Factor (BCF)—The quotient of the concentration of a chemical in aquatic organisms at a specific time or during a discrete time period of exposure divided by the concentration in the surrounding water at the same time or during the same period.

Biomarkers—Broadly defined as indicators signaling events in biologic systems or samples. They have been classified as markers of exposure, markers of effect, and markers of susceptibility.

Cancer Effect Level (CEL)—The lowest dose of chemical in a study, or group of studies, that produces significant increases in the incidence of cancer (or tumors) between the exposed population and its appropriate control.

Carcinogen—A chemical capable of inducing cancer.

Case-Control Study— A type of epidemiological study that examines the relationship between a particular outcome (disease or condition) and a variety of potential causative agents (such as toxic chemicals). In a case-control study, a group of people with a specified and well-defined outcome is identified and compared to a similar group of people without the outcome.

Case Report—Describes a single individual with a particular disease or exposure. These may suggest some potential topics for scientific research, but are not actual research studies.

Case Series—Describes the experience of a small number of individuals with the same disease or exposure. These may suggest potential topics for scientific research, but are not actual research studies.

Ceiling Value—A concentration that must not be exceeded.

Chronic Exposure—Exposure to a chemical for 365 days or more, as specified in the Toxicological Profiles.

Cohort Study—A type of epidemiological study of a specific group or groups of people who have had a common insult (e.g., exposure to an agent suspected of causing disease or a common disease) and are followed forward from exposure to outcome. At least one exposed group is compared to one unexposed group.

Cross-sectional Study—A type of epidemiological study of a group or groups of people that examines the relationship between exposure and outcome to a chemical or to chemicals at one point in time.

Data Needs—Substance-specific informational needs that, if met, would reduce the uncertainties of human health risk assessment.

Developmental Toxicity—The occurrence of adverse effects on the developing organism that may result from exposure to a chemical prior to conception (either parent), during prenatal development, or postnatally to the time of sexual maturation. Adverse developmental effects may be detected at any point in the life span of the organism.

Dose-Response Relationship—The quantitative relationship between the amount of exposure to a toxicant and the incidence of the adverse effects.

Embryotoxicity and Fetotoxicity—Any toxic effect on the conceptus as a result of prenatal exposure to a chemical; the distinguishing feature between the two terms is the stage of development during which the insult occurs. The terms, as used here, include malformations and variations, altered growth, and *in utero* death.

Environmental Protection Agency (EPA) Health Advisory—An estimate of acceptable drinking water levels for a chemical substance based on health effects information. A health advisory is not a legally enforceable federal standard, but serves as technical guidance to assist federal, state, and local officials.

Epidemiology—Refers to the investigation of factors that determine the frequency and distribution of disease or other health-related conditions within a defined human population during a specified period.

Genotoxicity—A specific adverse effect on the genome of living cells that, upon the duplication of affected cells, can be expressed as a mutagenic, clastogenic, or carcinogenic event because of specific alteration of the molecular structure of the genome.

Half-life—A measure of rate for the time required to eliminate one half of a quantity of a chemical from the body or environmental media.

Immediately Dangerous to Life or Health (IDLH)—A condition that poses a threat of life or health, or conditions that pose an immediate threat of severe exposure to contaminants that are likely to have adverse cumulative or delayed effects on health.

Immunologic Toxicity—The occurrence of adverse effects on the immune system that may result from exposure to environmental agents such as chemicals.

Immunological Effects—Functional changes in the immune response.

Incidence—The ratio of new cases of individuals in a population who develop a specified condition to the total number of individuals in that population who could have developed that condition in a specified time period.

Intermediate Exposure—Exposure to a chemical for a duration of 15–364 days, as specified in the Toxicological Profiles.

***In Vitro*—**Isolated from the living organism and artificially maintained, as in a test tube.

***In Vivo*—**Occurring within the living organism.

Lethal Concentration$_{(LO)}$ (LC_{LO})—The lowest concentration of a chemical in air that has been reported to have caused death in humans or animals.

Lethal Concentration$_{(50)}$ (LC_{50})—A calculated concentration of a chemical in air to which exposure for a specific length of time is expected to cause death in 50% of a defined experimental animal population.

Lethal Dose$_{(LO)}$ (LD_{Lo})—The lowest dose of a chemical introduced by a route other than inhalation that has been reported to have caused death in humans or animals.

Lethal Dose$_{(50)}$ (LD_{50})—The dose of a chemical that has been calculated to cause death in 50% of a defined experimental animal population.

Lethal Time$_{(50)}$ (LT_{50})—A calculated period of time within which a specific concentration of a chemical is expected to cause death in 50% of a defined experimental animal population.

Lowest-Observed-Adverse-Effect Level (LOAEL)—The lowest exposure level of chemical in a study, or group of studies, that produces statistically or biologically significant increases in frequency or severity of adverse effects between the exposed population and its appropriate control.

Lymphoreticular Effects—Represent morphological effects involving lymphatic tissues such as the lymph nodes, spleen, and thymus.

Malformations—Permanent structural changes that may adversely affect survival, development, or function.

Minimal Risk Level (MRL)—An estimate of daily human exposure to a hazardous substance that is likely to be without an appreciable risk of adverse noncancer health effects over a specified route and duration of exposure.

Modifying Factor (MF)—A value (greater than zero) that is applied to the derivation of a Minimal Risk Level (MRL) to reflect additional concerns about the database that are not covered by the uncertainty factors. The default value for a MF is 1.

Morbidity—State of being diseased; morbidity rate is the incidence or prevalence of disease in a specific population.

Mortality—Death; mortality rate is a measure of the number of deaths in a population during a specified interval of time.

Mutagen—A substance that causes mutations. A mutation is a change in the DNA sequence of a cell's DNA. Mutations can lead to birth defects, miscarriages, or cancer.

Necropsy—The gross examination of the organs and tissues of a dead body to determine the cause of death or pathological conditions.

Neurotoxicity—The occurrence of adverse effects on the nervous system following exposure to a hazardous substance.

No-Observed-Adverse-Effect Level (NOAEL)—The dose of a chemical at which there were no statistically or biologically significant increases in frequency or severity of adverse effects seen between the exposed population and its appropriate control. Effects may be produced at this dose, but they are not considered to be adverse.

Octanol-Water Partition Coefficient (K_{ow})—The equilibrium ratio of the concentrations of a chemical in *n*-octanol and water, in dilute solution.

Odds Ratio (OR)—A means of measuring the association between an exposure (such as toxic substances and a disease or condition) that represents the best estimate of relative risk (risk as a ratio of the incidence among subjects exposed to a particular risk factor divided by the incidence among subjects who were not exposed to the risk factor). An OR of greater than 1 is considered to indicate greater risk of disease in the exposed group compared to the unexposed group.

Organophosphate or Organophosphorus Compound—A phosphorus-containing organic compound and especially a pesticide that acts by inhibiting cholinesterase.

Permissible Exposure Limit (PEL)—An Occupational Safety and Health Administration (OSHA) regulatory limit on the amount or concentration of a substance not to be exceeded in workplace air averaged over any 8-hour work shift of a 40-hour workweek.

Pesticide—General classification of chemicals specifically developed and produced for use in the control of agricultural and public health pests (insects or other organisms harmful to cultivated plants or animals).

Pharmacokinetics—The dynamic behavior of a material in the body, used to predict the fate (disposition) of an exogenous substance in an organism. Utilizing computational techniques, it provides the means of studying the absorption, distribution, metabolism, and excretion of chemicals by the body.

Pharmacokinetic Model—A set of equations that can be used to describe the time course of a parent chemical or metabolite in an animal system. There are two types of pharmacokinetic models: data-based and physiologically-based. A data-based model divides the animal system into a series of compartments, which, in general, do not represent real, identifiable anatomic regions of the body, whereas the physiologically-based model compartments represent real anatomic regions of the body.

Physiologically Based Pharmacodynamic (PBPD) Model—A type of physiologically based dose-response model that quantitatively describes the relationship between target tissue dose and toxic end points. These models advance the importance of physiologically based models in that they clearly describe the biological effect (response) produced by the system following exposure to an exogenous substance.

Physiologically Based Pharmacokinetic (PBPK) Model—Comprised of a series of compartments representing organs or tissue groups with realistic weights and blood flows. These models require a variety of physiological information: tissue volumes, blood flow rates to tissues, cardiac output, alveolar ventilation rates, and possibly membrane permeabilities. The models also utilize biochemical information, such as blood:air partition coefficients, and metabolic parameters. PBPK models are also called biologically based tissue dosimetry models.

Prevalence—The number of cases of a disease or condition in a population at one point in time.

Prospective Study—A type of cohort study in which the pertinent observations are made on events occurring after the start of the study. A group is followed over time.

q_1*—The upper-bound estimate of the low-dose slope of the dose-response curve as determined by the multistage procedure. The q_1* can be used to calculate an estimate of carcinogenic potency, the incremental excess cancer risk per unit of exposure (usually µg/L for water, mg/kg/day for food, and µg/m^3 for air).

Recommended Exposure Limit (REL)—A National Institute for Occupational Safety and Health (NIOSH) time-weighted average (TWA) concentration for up to a 10-hour workday during a 40-hour workweek.

Reference Concentration (RfC)—An estimate (with uncertainty spanning perhaps an order of magnitude) of a continuous inhalation exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious noncancer health effects during a lifetime. The inhalation reference concentration is for continuous inhalation exposures and is appropriately expressed in units of mg/m^3 or ppm.

Reference Dose (RfD)—An estimate (with uncertainty spanning perhaps an order of magnitude) of the daily exposure of the human population to a potential hazard that is likely to be without risk of deleterious effects during a lifetime. The RfD is operationally derived from the no-observed-adverse-effect level (NOAEL, from animal and human studies) by a consistent application of uncertainty factors that reflect various types of data used to estimate RfDs and an additional modifying factor, which is based on a professional judgment of the entire database on the chemical. The RfDs are not applicable to nonthreshold effects such as cancer.

Reportable Quantity (RQ)—The quantity of a hazardous substance that is considered reportable under the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA). Reportable quantities are (1) 1 pound or greater or (2) for selected substances, an amount established by regulation either under CERCLA or under Section 311 of the Clean Water Act. Quantities are measured over a 24-hour period.

Reproductive Toxicity—The occurrence of adverse effects on the reproductive system that may result from exposure to a hazardous substance. The toxicity may be directed to the reproductive organs and/or the related endocrine system. The manifestation of such toxicity may be noted as alterations in sexual behavior, fertility, pregnancy outcomes, or modifications in other functions that are dependent on the integrity of this system.

Retrospective Study—A type of cohort study based on a group of persons known to have been exposed at some time in the past. Data are collected from routinely recorded events, up to the time the study is undertaken. Retrospective studies are limited to causal factors that can be ascertained from existing records and/or examining survivors of the cohort.

Risk—The possibility or chance that some adverse effect will result from a given exposure to a hazardous substance.

Risk Factor—An aspect of personal behavior or lifestyle, an environmental exposure, existing health condition, or an inborn or inherited characteristic that is associated with an increased occurrence of disease or other health-related event or condition.

Risk Ratio—The ratio of the risk among persons with specific risk factors compared to the risk among persons without risk factors. A risk ratio greater than 1 indicates greater risk of disease in the exposed group compared to the unexposed group.

Short-Term Exposure Limit (STEL)—A STEL is a 15-minute TWA exposure that should not be exceeded at any time during a workday.

Standardized Mortality Ratio (SMR)—A ratio of the observed number of deaths and the expected number of deaths in a specific standard population.

Target Organ Toxicity—This term covers a broad range of adverse effects on target organs or physiological systems (e.g., renal, cardiovascular) extending from those arising through a single limited exposure to those assumed over a lifetime of exposure to a chemical.

Teratogen—A chemical that causes structural defects that affect the development of an organism.

Threshold Limit Value (TLV)—An American Conference of Governmental Industrial Hygienists (ACGIH) concentration of a substance to which it is believed that nearly all workers may be repeatedly exposed, day after day, for a working lifetime without adverse effect. The TLV may be expressed as a Time Weighted Average (TLV-TWA), as a Short-Term Exposure Limit (TLV-STEL), or as a ceiling limit (TLV-C).

Time-Weighted Average (TWA)—An average exposure within a given time period.

Toxic Dose$_{(50)}$ (TD$_{50}$)—A calculated dose of a chemical, introduced by a route other than inhalation, which is expected to cause a specific toxic effect in 50% of a defined experimental animal population.

Toxicokinetic—The absorption, distribution, and elimination of toxic compounds in the living organism.

Uncertainty Factor (UF)—A factor used in operationally deriving the Minimal Risk Level (MRL) or Reference Dose (RfD) or Reference Concentration (RfC) from experimental data. UFs are intended to account for (1) the variation in sensitivity among the members of the human population, (2) the uncertainty in extrapolating animal data to the case of human, (3) the uncertainty in extrapolating from data obtained in a study that is of less than lifetime exposure, and (4) the uncertainty in using lowest-observed-adverse-effect level (LOAEL) data rather than no-observed-adverse-effect level (NOAEL) data. A default for each individual UF is 10; if complete certainty in data exists, a value of 1 can be used; however, a reduced UF of 3 may be used on a case-by-case basis, 3 being the approximate logarithmic average of 10 and 1.

Xenobiotic—Any substance that is foreign to the biological system.

APPENDIX A. ATSDR MINIMAL RISK LEVELS AND WORKSHEETS

The Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) [42 U.S.C. 9601 et seq.], as amended by the Superfund Amendments and Reauthorization Act (SARA) [Pub. L. 99–499], requires that the Agency for Toxic Substances and Disease Registry (ATSDR) develop jointly with the U.S. Environmental Protection Agency (EPA), in order of priority, a list of hazardous substances most commonly found at facilities on the CERCLA National Priorities List (NPL); prepare toxicological profiles for each substance included on the priority list of hazardous substances; and assure the initiation of a research program to fill identified data needs associated with the substances.

The toxicological profiles include an examination, summary, and interpretation of available toxicological information and epidemiologic evaluations of a hazardous substance. During the development of toxicological profiles, Minimal Risk Levels (MRLs) are derived when reliable and sufficient data exist to identify the target organ(s) of effect or the most sensitive health effect(s) for a specific duration for a given route of exposure. An MRL is an estimate of the daily human exposure to a hazardous substance that is likely to be without appreciable risk of adverse noncancer health effects over a specified route and duration of exposure. MRLs are based on noncancer health effects only and are not based on a consideration of cancer effects. These substance-specific estimates, which are intended to serve as screening levels, are used by ATSDR health assessors to identify contaminants and potential health effects that may be of concern at hazardous waste sites. It is important to note that MRLs are not intended to define clean-up or action levels.

MRLs are derived for hazardous substances using the no-observed-adverse-effect level/uncertainty factor approach. They are below levels that might cause adverse health effects in the people most sensitive to such chemical-induced effects. MRLs are derived for acute (1–14 days), intermediate (15–364 days), and chronic (365 days and longer) durations and for the oral and inhalation routes of exposure. Currently, MRLs for the dermal route of exposure are not derived because ATSDR has not yet identified a method suitable for this route of exposure. MRLs are generally based on the most sensitive substance-induced endpoint considered to be of relevance to humans. Serious health effects (such as irreparable damage to the liver or kidneys, or birth defects) are not used as a basis for establishing MRLs. Exposure to a level above the MRL does not mean that adverse health effects will occur.

MRLs are intended only to serve as a screening tool to help public health professionals decide where to look more closely. They may also be viewed as a mechanism to identify those hazardous waste sites that

are not expected to cause adverse health effects. Most MRLs contain a degree of uncertainty because of the lack of precise toxicological information on the people who might be most sensitive (e.g., infants, elderly, nutritionally or immunologically compromised) to the effects of hazardous substances. ATSDR uses a conservative (i.e., protective) approach to address this uncertainty consistent with the public health principle of prevention. Although human data are preferred, MRLs often must be based on animal studies because relevant human studies are lacking. In the absence of evidence to the contrary, ATSDR assumes that humans are more sensitive to the effects of hazardous substance than animals and that certain persons may be particularly sensitive. Thus, the resulting MRL may be as much as 100-fold below levels that have been shown to be nontoxic in laboratory animals.

Proposed MRLs undergo a rigorous review process: Health Effects/MRL Workgroup reviews within the Division of Toxicology and Human Health Sciences, expert panel peer reviews, and agency-wide MRL Workgroup reviews, with participation from other federal agencies and comments from the public. They are subject to change as new information becomes available concomitant with updating the toxicological profiles. Thus, MRLs in the most recent toxicological profiles supersede previously published levels. For additional information regarding MRLs, please contact the Division of Toxicology and Human Health Sciences, Agency for Toxic Substances and Disease Registry, 1600 Clifton Road NE, Mailstop F-57, Atlanta, Georgia 30329-4027.

APPENDIX A

MINIMAL RISK LEVEL (MRL) WORKSHEET

Chemical Name: 2,4-D
CAS Numbers: 94-75-7
Date: February 2017
Profile Status: Final Pre-Public Comment
Route: [ ] Inhalation [X] Oral
Duration: [ ] Acute [X] Intermediate [ ] Chronic
Graph Key: 98
Species: Rats

Minimal Risk Level: 0.009 [X] mg/kg/day [ ] ppm

Reference: Stürtz N, Jahn GA, Deis RP, et al. 2010. Effect of 2,4-dichlorophenoxyacetic acid on milk transfer to the litter and prolactin release in lactating rats. Toxicology 271(1-2):13-20.

Experimental design: Groups of female Wistar rats (6–8/group) were fed a diet that provided 0, 2.5, 5, 10, 15, 25, 50, or 70 mg/kg/day 2,4-D (98% pure) on postpartum days 1–16. Dams were checked daily for clinical signs, and food consumption and body weight were monitored. Milk ejection was assessed by changes in body weight of the pups after allowing the pups to suckle during 15-minute periods on postpartum days 11–13. Blood was collected from the dams on postpartum day 12 for determination of growth hormone, prolactin, and oxytocin. Dams were sacrificed on postpartum day 16, and the arcuate nucleus and the anterior lobe of the pituitary were isolated for biochemical analyses of monoamines and metabolites in the 15, 25, and 50 mg/kg/day dose groups.

Effect noted in study and corresponding doses: Exposure to 2,4-D did not affect maternal body weight, and no pups died during the test period. Exposure to 2,4-D significantly reduced pup weight beginning on postnatal day (PND) 7 in all exposed groups except the lowest dose group; this group showed a significant reduction in body weight beginning on PND 10. Milk ejection was significantly reduced in all treated groups on postpartum day 13 by >50%, reaching approximately 75% reduction in the highest dose group. However, there were no significant differences between the lowest four treated groups (2.5, 5, 10, and 15 mg/kg/day groups). An injection of oxytocin to the dams partially restored milk production, indicating that 2,4-D, at least in part, inhibited oxytocin release, but not the capacity of the mammary gland to produce or secrete milk. Serum prolactin appeared to be reduced in all treated groups, although Figure 3A in the study does not indicate statistically significant differences between the controls and exposed groups. Serum oxytocin was significantly reduced at ≥25 mg 2,4-D/kg/day. Serotonin was significantly reduced in the arcuate nucleus at ≥15 mg 2,4-D/kg/day and dopamine was significantly increased at ≥25 mg/kg/day. Dopamine was also increased in the anterior pituitary at ≥15 mg 2,4-D/kg/day.

The offspring body weight data on PND 16 (Table A-1) were fit to all available continuous models in EPA's Benchmark Dose Software (BMDS) version 2.4.0 using a BMR of 5% change from control.

Table A-1. Dataset for Offspring Weight on Postnatal Day 16[a]

Dose (mg/kg/day)	Litter number	Mean pup weight (g)	Standard deviation
0	12	30.1	0.3
2.5	8	27.9[b]	0.8
5.0	8	26.9[b]	0.8
15.0	8	26.7[b]	0.8
25.0	8	26.6[b]	0.6
50.0	8	24.7[b]	0.6
70.0	8	25.1[b]	0.8

[a]Data from Table 1 in Stürtz et al. (2010).
[b]$p<0.001$.

Although there are no established guidelines as to what minimal change in a continuous end point such as body weight is biologically significant, a 10% change is generally used for adult body weight. However, because fetal or neonatal organisms may be more susceptible than adults, a 5% change was deemed appropriate. The following procedure for fitting continuous data was used. The simplest model (linear) was first applied to the data while assuming constant variance. If the data were consistent with the assumption of constant variance ($p \geq 0.1$), then the fit of the linear model to the means was evaluated and the polynomial, power, exponential, and Hill models were fit to the data while assuming constant variance. Adequate model fit was judged by three criteria: goodness-of-fit p-value ($p>0.1$), visual inspection of the dose-response curve, and scaled residual at the data point (except the control) closest to the predefined BMR. Among all of the models providing adequate fit to the data, the lowest BMDL (95% lower confidence limit on BMC) was selected as the POD when the difference between the BMCLs estimated from these models were >3-fold; otherwise, the BMCL from the model with the lowest AIC was chosen. If the test for constant variance was negative, the linear model was run again while applying the power model integrated into the BMCS to account for nonhomogenous variance. If the nonhomogenous variance model provided an adequate fit ($p \geq 0.1$) to the variance data, then the fit of the linear model to the means was evaluated and the polynomial, power, exponential, and Hill models were fit to the data and evaluated while the variance model was applied. Model fit and POD selection proceeded as described earlier. If the test for constant variance was negative and the nonhomogenous variance model did not provide an adequate fit to the variance data, then the data set was considered unsuitable for modeling.

Because no models fit the complete dataset, first the highest dose and subsequently the next highest dose were dropped.

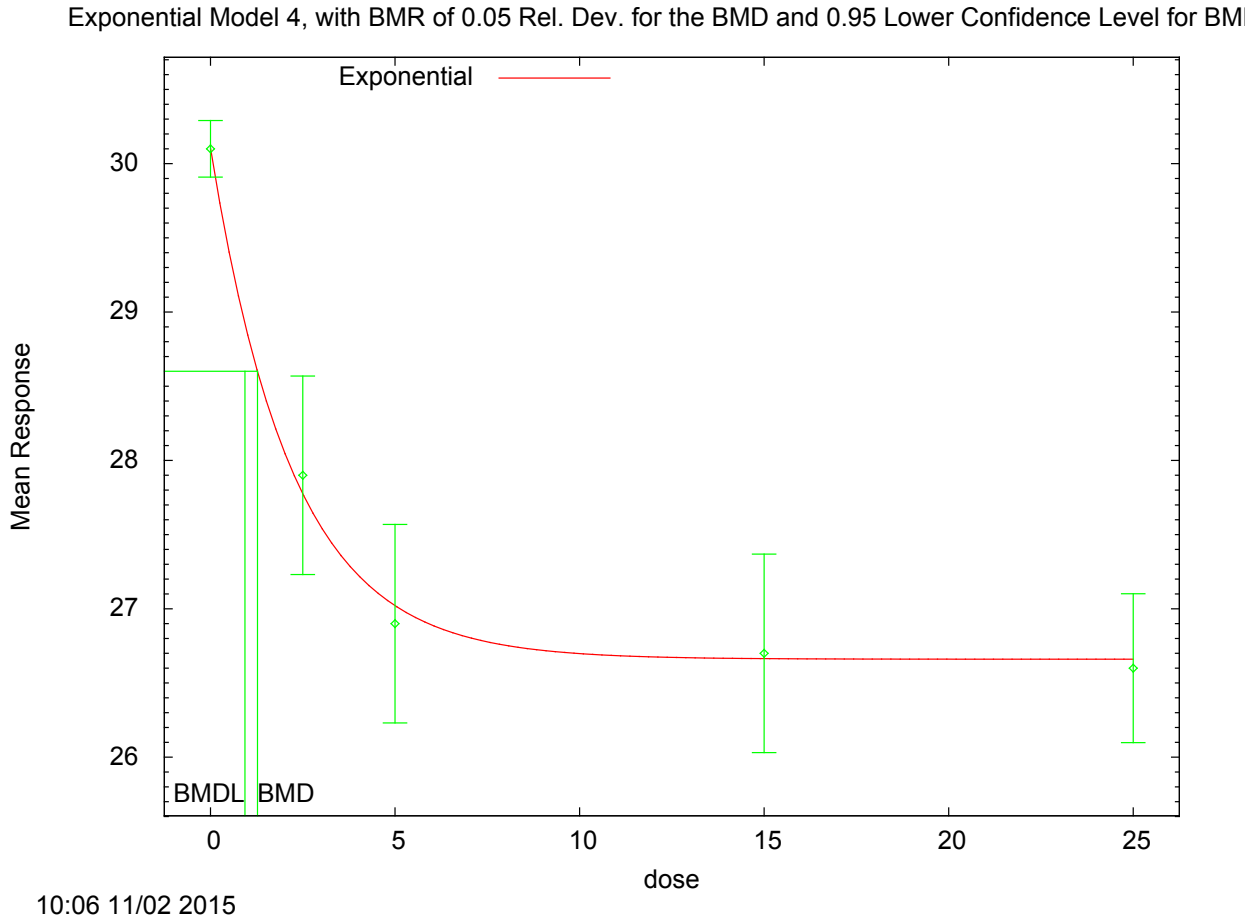
As seen in Table A-2, only two BMD models (Exponential model 4 and Hill model) provided an adequate fit by the various statistical criteria. Because the $BMDL_{RD05}$ estimates are sufficiently close, the model with the lowest AIC (Exponential model 4) was selected. The Exponential model calculated BMD_{RD05} and $BMDL_{RD05}$ values of 1.27 and 0.93 mg/kg/day, respectively, for decreased pup body weight on PND 16 (see Figure A-1). Dividing the $BMDL_{RD05}$ of 0.93 mg/kg/day by an uncertainty factor of 100 (10 for animal to human extrapolation and 10 for human variability) yields an intermediate-duration oral MRL of 0.009 mg/kg/day for 2,4-D.

Table A-2. Model predictions for Decreased Pup Body Weight Gain on Postnatal Day 16 (Stürtz et al. 2010)

Model	p-value[a] Test 1: lack dose response?	p-value[b] Test 3: good variance model?	p-value[b] for fit: does the model for the mean fit?	Scaled residuals[c] Dose below BMD	Scaled residuals[c] Dose above BMD	Scaled residuals[c] Overall largest	AIC	BMD_{RD05} (mg/kg/day)	$BMDL_{RD05}$ (mg/kg/day)
All doses									
Constant variance									
Linear[d]	<0.0001	0.06	<0.0001	-1.91	-0.73	4.83	88.54	23.63	20.27
Nonconstant variance									
Linear[d]	<0.0001	0.16	<0.0001	-0.75	-2.41	4.66	88.12	25.98	21.72
High dose dropped									
Constant variance									
Linear[d]	<0.0001	0.05	<0.0001	-1.71	0.20	4.44	69.97	16.72	14.32
Nonconstant variance									
Linear[d]	<0.0001	0.097	<0.0001	-1.73	0.14	4.10	61.85	18.57	16.46
Two highest doses dropped									
Constant variance									
Linear[d]	<0.0001	0.03	<0.0001	-3.37	-1.07	3.74	62.83	12.64	9.99
Nonconstant variance									
Exponential (model 2)[e]	<0.0001	0.35	<0.0001	-1.61	1.19	3.65	59.58	15.38	11.69
Exponential (model 3)[e]	<0.0001	0.35	<0.0001	-1.61	1.19	3.65	59.58	15.38	11.69
Exponential (model 4)[e,f]	***<0.0001***	***0.35***	***0.82***	***-0.07***	***0.61***	***0.61***	***4.60***	***1.27***	***0.93***
Exponential (model 5)[e]	<0.0001	0.35	0.0008	-1.02×10^{-6}	2.83	2.83	17.51	0.73	1.07×10^{-3}
Hill[e]	<0.0001	0.35	0.95	-0.05	0.32	-0.33	6.21	1.83	0.70
Linear[d]	<0.0001	0.35	<0.0001	-1.65	1.16	3.69	60.05	15.75	12.23
Polynomial (2-degree)[d]	<0.0001	0.35	<0.0001	-1.65	1.16	3.69	60.05	15.75	12.23
Polynomial (3-degree)[d]	<0.0001	0.35	<0.0001	-1.65	1.16	3.69	60.05	15.75	12.23
Polynomial (4-degree)[d]	<0.0001	0.35	<0.0001	-1.65	1.16	3.69	60.05	15.75	12.23
Power[e]	<0.0001	0.35	<0.0001	-1.65	1.16	3.69	58.05	15.75	12.23

[a]Values >0.05 fail to meet conventional goodness-of-fit criteria.
[b]Values <0.10 fail to meet conventional goodness-of-fit criteria.
[c]Scaled residuals at doses immediately below and above the benchmark dose; also the largest residual at any dose.
[d]Coefficients restricted to be negative.
[e]Power restricted to ≥1.
[f]Selected model. No models fit the full dataset. With the two highest doses dropped, the nonconstant variance models fit the variance data and only two models, Exponential model 4 and the Hill model, were fit to the means. BMDLs for models providing adequate fit were sufficiently close (differed by <2–3-fold), so the model with the lowest AIC was selected (Exponential model 4).
AIC = Akaike Information Criterion; BMD = maximum likelihood estimate of the exposure concentration associated with the selected benchmark response; BMDL = 95% lower confidence limit on the BMD (subscripts denote benchmark response: i.e., $_{05}$ = dose associated with 5% extra risk); RD = relative deviation

Figure 1. Selected Model (Exponential Model 4) for Decreased Pup Body Weight on Postnatal Day 16 (Stürtz et al. 2010)



Dose and end point used for MRL derivation: Decreased offspring body weight on PND 16 at maternal doses of 0–25 mg 2,4-D/kg/day on postpartum days 1–16. Modeling used dose ranges from 0 to 25 mg/kg/day. The POD was 2.5 mg/kg/day.

[ ] NOAEL [ ] LOAEL [X] $BMDL_{RD05}$

Uncertainty Factors used in MRL derivation:

[ ] 10 for use of a LOAEL
[X] 10 for extrapolation from animals to humans
[X] 10 for human variability

Was a conversion factor used from ppm in food or water to a mg/body weight dose? No.

If an inhalation study in animals, list conversion factors used in determining human equivalent dose: Not applicable.

Was a conversion used from intermittent to continuous exposure? No.

Other additional studies or pertinent information that lend support to this MRL: Reduced offspring body weight was also reported in other studies in which rat dams were exposed to 2,4-D for longer periods that also included postpartum, although at higher estimated maternal doses of 2,4-D. For example, in a 2-generation reproductive study, pup body weight was reduced significantly on PND 28 at estimated maternal doses ≥35 mg 2,4-D/kg/day during lactation, but not at 10 mg 2,4-D/kg/day (EPA 1986). Marty et al. (2013) reported significantly reduced pup weight (about 10%) on PND 22 at estimated maternal doses of approximately 9 mg 2,4-D/kg/day during lactation, but lower doses were not tested. In a 3-generation study, reduced pup weight was noted at maternal doses of approximately 111 mg 2,4-D/kg/day, but not 37 mg/kg/day (Hansen et al. 1971). The reasons for the apparent discrepancy regarding maternal dose levels at which offspring weight is significantly affected are not clear, but could be related to the different manners of estimating maternal intake of test material. Other studies that reported reduced offspring weight at higher maternal 2,4-D doses include Bortolozzi et al. (1999), Mazhar et al. (2014), and Troudi et al. (2012a, 2012b). While there seems to be some discrepancy between the results of these developmental studies with regard to fetal weight, there does not seem to be a good reason to discount the results of Stürtz et al. (2010).

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APPENDIX B. USER'S GUIDE

Chapter 1

Public Health Statement

This chapter of the profile is a health effects summary written in non-technical language. Its intended audience is the general public, especially people living in the vicinity of a hazardous waste site or chemical release. If the Public Health Statement were removed from the rest of the document, it would still communicate to the lay public essential information about the chemical.

The major headings in the Public Health Statement are useful to find specific topics of concern. The topics are written in a question and answer format. The answer to each question includes a sentence that will direct the reader to chapters in the profile that will provide more information on the given topic.

Chapter 2

Relevance to Public Health

This chapter provides a health effects summary based on evaluations of existing toxicologic, epidemiologic, and toxicokinetic information. This summary is designed to present interpretive, weight-of-evidence discussions for human health end points by addressing the following questions:

1. What effects are known to occur in humans?

2. What effects observed in animals are likely to be of concern to humans?

3. What exposure conditions are likely to be of concern to humans, especially around hazardous waste sites?

The chapter covers end points in the same order that they appear within the Discussion of Health Effects by Route of Exposure section, by route (inhalation, oral, and dermal) and within route by effect. Human data are presented first, then animal data. Both are organized by duration (acute, intermediate, chronic). *In vitro* data and data from parenteral routes (intramuscular, intravenous, subcutaneous, etc.) are also considered in this chapter.

The carcinogenic potential of the profiled substance is qualitatively evaluated, when appropriate, using existing toxicokinetic, genotoxic, and carcinogenic data. ATSDR does not currently assess cancer potency or perform cancer risk assessments. Minimal Risk Levels (MRLs) for noncancer end points (if derived) and the end points from which they were derived are indicated and discussed.

Limitations to existing scientific literature that prevent a satisfactory evaluation of the relevance to public health are identified in the Chapter 3 Data Needs section.

Interpretation of Minimal Risk Levels

Where sufficient toxicologic information is available, ATSDR has derived MRLs for inhalation and oral routes of entry at each duration of exposure (acute, intermediate, and chronic). These MRLs are not meant to support regulatory action, but to acquaint health professionals with exposure levels at which adverse health effects are not expected to occur in humans.

MRLs should help physicians and public health officials determine the safety of a community living near a hazardous substance emission, given the concentration of a contaminant in air or the estimated daily dose in water. MRLs are based largely on toxicological studies in animals and on reports of human occupational exposure.

MRL users should be familiar with the toxicologic information on which the number is based. Chapter 2, "Relevance to Public Health," contains basic information known about the substance. Other sections such as Chapter 3 Section 3.9, "Interactions with Other Substances," and Section 3.10, "Populations that are Unusually Susceptible" provide important supplemental information.

MRL users should also understand the MRL derivation methodology. MRLs are derived using a modified version of the risk assessment methodology that the Environmental Protection Agency (EPA) provides (Barnes and Dourson 1988) to determine reference doses (RfDs) for lifetime exposure.

To derive an MRL, ATSDR generally selects the most sensitive end point which, in its best judgement, represents the most sensitive human health effect for a given exposure route and duration. ATSDR cannot make this judgement or derive an MRL unless information (quantitative or qualitative) is available for all potential systemic, neurological, and developmental effects. If this information and reliable quantitative data on the chosen end point are available, ATSDR derives an MRL using the most sensitive species (when information from multiple species is available) with the highest no-observed-adverse-effect level (NOAEL) that does not exceed any adverse effect levels. When a NOAEL is not available, a lowest-observed-adverse-effect level (LOAEL) can be used to derive an MRL, and an uncertainty factor (UF) of 10 must be employed. Additional uncertainty factors of 10 must be used both for human variability to protect sensitive subpopulations (people who are most susceptible to the health effects caused by the substance) and for interspecies variability (extrapolation from animals to humans). In deriving an MRL, these individual uncertainty factors are multiplied together. The product is then divided into the inhalation concentration or oral dosage selected from the study. Uncertainty factors used in developing a substance-specific MRL are provided in the footnotes of the levels of significant exposure (LSE) tables.

Chapter 3

Health Effects

Tables and Figures for Levels of Significant Exposure (LSE)

Tables and figures are used to summarize health effects and illustrate graphically levels of exposure associated with those effects. These levels cover health effects observed at increasing dose concentrations and durations, differences in response by species, MRLs to humans for noncancer end points, and EPA's estimated range associated with an upper- bound individual lifetime cancer risk of 1 in 10,000 to 1 in 10,000,000. Use the LSE tables and figures for a quick review of the health effects and to locate data for a specific exposure scenario. The LSE tables and figures should always be used in conjunction with the text. All entries in these tables and figures represent studies that provide reliable, quantitative estimates of NOAELs, LOAELs, or Cancer Effect Levels (CELs).

The legends presented below demonstrate the application of these tables and figures. Representative examples of LSE Table 3-1 and Figure 3-1 are shown. The numbers in the left column of the legends correspond to the numbers in the example table and figure.

LEGEND

See Sample LSE Table 3-1 (page B-6)

(1) Route of Exposure. One of the first considerations when reviewing the toxicity of a substance using these tables and figures should be the relevant and appropriate route of exposure. Typically when sufficient data exist, three LSE tables and two LSE figures are presented in the document. The three LSE tables present data on the three principal routes of exposure, i.e., inhalation, oral, and dermal (LSE Tables 3-1, 3-2, and 3-3, respectively). LSE figures are limited to the inhalation (LSE Figure 3-1) and oral (LSE Figure 3-2) routes. Not all substances will have data on each route of exposure and will not, therefore, have all five of the tables and figures.

(2) Exposure Period. Three exposure periods—acute (less than 15 days), intermediate (15–364 days), and chronic (365 days or more)—are presented within each relevant route of exposure. In this example, an inhalation study of intermediate exposure duration is reported. For quick reference to health effects occurring from a known length of exposure, locate the applicable exposure period within the LSE table and figure.

(3) Health Effect. The major categories of health effects included in LSE tables and figures include death, systemic, immunological, neurological, developmental, reproductive, and cancer. NOAELs and LOAELs can be reported in the tables and figures for all effects but cancer. Systemic effects are further defined in the "System" column of the LSE table (see key number 18).

(4) Key to Figure. Each key number in the LSE table links study information to one or more data points using the same key number in the corresponding LSE figure. In this example, the study represented by key number 18 has been used to derive a NOAEL and a Less Serious LOAEL (also see the two "18r" data points in sample Figure 3-1).

(5) Species. The test species, whether animal or human, are identified in this column. Chapter 2, "Relevance to Public Health," covers the relevance of animal data to human toxicity and Section 3.4, "Toxicokinetics," contains any available information on comparative toxicokinetics. Although NOAELs and LOAELs are species specific, the levels are extrapolated to equivalent human doses to derive an MRL.

(6) Exposure Frequency/Duration. The duration of the study and the weekly and daily exposure regimens are provided in this column. This permits comparison of NOAELs and LOAELs from different studies. In this case (key number 18), rats were exposed to “Chemical x” via inhalation for 6 hours/day, 5 days/week, for 13 weeks. For a more complete review of the dosing regimen, refer to the appropriate sections of the text or the original reference paper (i.e., Nitschke et al. 1981).

(7) System. This column further defines the systemic effects. These systems include respiratory, cardiovascular, gastrointestinal, hematological, musculoskeletal, hepatic, renal, and dermal/ocular. "Other" refers to any systemic effect (e.g., a decrease in body weight) not covered in these systems. In the example of key number 18, one systemic effect (respiratory) was investigated.

(8) NOAEL. A NOAEL is the highest exposure level at which no adverse effects were seen in the organ system studied. Key number 18 reports a NOAEL of 3 ppm for the respiratory system, which was used to derive an intermediate exposure, inhalation MRL of 0.005 ppm (see footnote "b").

(9) LOAEL. A LOAEL is the lowest dose used in the study that caused an adverse health effect. LOAELs have been classified into "Less Serious" and "Serious" effects. These distinctions help readers identify the levels of exposure at which adverse health effects first appear and the gradation of effects with increasing dose. A brief description of the specific end point used to quantify the adverse effect accompanies the LOAEL. The respiratory effect reported in key number 18 (hyperplasia) is a Less Serious LOAEL of 10 ppm. MRLs are not derived from Serious LOAELs.

(10) Reference. The complete reference citation is given in Chapter 9 of the profile.

(11) CEL. A CEL is the lowest exposure level associated with the onset of carcinogenesis in experimental or epidemiologic studies. CELs are always considered serious effects. The LSE tables and figures do not contain NOAELs for cancer, but the text may report doses not causing measurable cancer increases.

(12) Footnotes. Explanations of abbreviations or reference notes for data in the LSE tables are found in the footnotes. Footnote "b" indicates that the NOAEL of 3 ppm in key number 18 was used to derive an MRL of 0.005 ppm.

LEGEND

See Sample Figure 3-1 (page B-7)

LSE figures graphically illustrate the data presented in the corresponding LSE tables. Figures help the reader quickly compare health effects according to exposure concentrations for particular exposure periods.

(13) Exposure Period. The same exposure periods appear as in the LSE table. In this example, health effects observed within the acute and intermediate exposure periods are illustrated.

(14) Health Effect. These are the categories of health effects for which reliable quantitative data exists. The same health effects appear in the LSE table.

(15) Levels of Exposure. Concentrations or doses for each health effect in the LSE tables are graphically displayed in the LSE figures. Exposure concentration or dose is measured on the log scale "y" axis. Inhalation exposure is reported in mg/m^3 or ppm and oral exposure is reported in mg/kg/day.

(16) NOAEL. In this example, the open circle designated 18r identifies a NOAEL critical end point in the rat upon which an intermediate inhalation exposure MRL is based. The key number 18 corresponds to the entry in the LSE table. The dashed descending arrow indicates the extrapolation from the exposure level of 3 ppm (see entry 18 in the table) to the MRL of 0.005 ppm (see footnote "b" in the LSE table).

(17) CEL. Key number 38m is one of three studies for which CELs were derived. The diamond symbol refers to a CEL for the test species-mouse. The number 38 corresponds to the entry in the LSE table.

(18) Estimated Upper-Bound Human Cancer Risk Levels. This is the range associated with the upper-bound for lifetime cancer risk of 1 in 10,000 to 1 in 10,000,000. These risk levels are derived from the EPA's Human Health Assessment Group's upper-bound estimates of the slope of the cancer dose response curve at low dose levels (q_1*).

(19) Key to LSE Figure. The Key explains the abbreviations and symbols used in the figure.

SAMPLE

1 → **Table 3-1. Levels of Significant Exposure to [Chemical x] – Inhalation**

Key to figure[a]	Species	Exposure frequency/ duration	System	NOAEL (ppm)	LOAEL (effect) Less serious (ppm)	LOAEL (effect) Serious (ppm)	Reference
2 → INTERMEDIATE EXPOSURE							
	5	6	7	8	9		10
3 → Systemic	↓	↓	↓	↓	↓		↓
18	Rat	13 wk 5 d/wk 6 hr/d	Resp	3[b]	10 (hyperplasia)		Nitschke et al. 1981
4 → CHRONIC EXPOSURE							
Cancer					11		
					↓		
38	Rat	18 mo 5 d/wk 7 hr/d			20	(CEL, multiple organs)	Wong et al. 1982
39	Rat	89–104 wk 5 d/wk 6 hr/d			10	(CEL, lung tumors, nasal tumors)	NTP 1982
40	Mouse	79–103 wk 5 d/wk 6 hr/d			10	(CEL, lung tumors, hemangiosarcomas)	NTP 1982

12 → [a] The number corresponds to entries in Figure 3-1.
[b] Used to derive an intermediate inhalation Minimal Risk Level (MRL) of $5x10^{-3}$ ppm; dose adjusted for intermittent exposure and divided by an uncertainty factor of 100 (10 for extrapolation from animal to humans, 10 for human variability).

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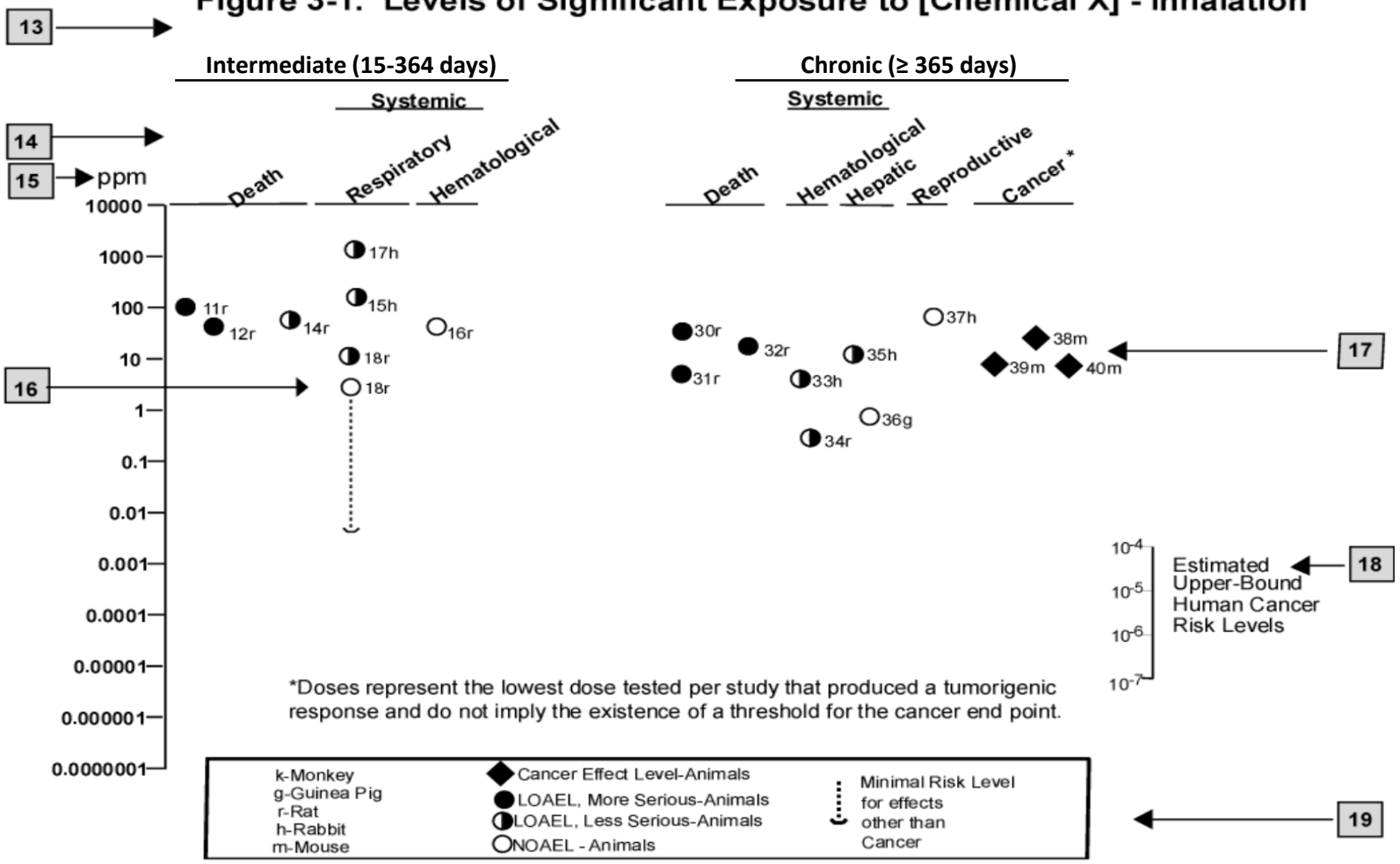
SAMPLE

Figure 3-1. Levels of Significant Exposure to [Chemical X] - Inhalation



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APPENDIX C. ACRONYMS, ABBREVIATIONS, AND SYMBOLS

ACGIH	American Conference of Governmental Industrial Hygienists
ACOEM	American College of Occupational and Environmental Medicine
ADI	acceptable daily intake
ADME	absorption, distribution, metabolism, and excretion
AED	atomic emission detection
AFID	alkali flame ionization detector
AFOSH	Air Force Office of Safety and Health
ALT	alanine aminotransferase
AML	acute myeloid leukemia
AOAC	Association of Official Analytical Chemists
AOEC	Association of Occupational and Environmental Clinics
AP	alkaline phosphatase
APHA	American Public Health Association
AST	aspartate aminotransferase
atm	atmosphere
ATSDR	Agency for Toxic Substances and Disease Registry
AWQC	Ambient Water Quality Criteria
BAT	best available technology
BCF	bioconcentration factor
BEI	Biological Exposure Index
BMD/C	benchmark dose or benchmark concentration
BMD_X	dose that produces a X% change in response rate of an adverse effect
$BMDL_X$	95% lower confidence limit on the BMD_X
BMDS	Benchmark Dose Software
BMR	benchmark response
BSC	Board of Scientific Counselors
C	centigrade
CAA	Clean Air Act
CAG	Cancer Assessment Group of the U.S. Environmental Protection Agency
CAS	Chemical Abstract Services
CDC	Centers for Disease Control and Prevention
CEL	cancer effect level
CELDS	Computer-Environmental Legislative Data System
CERCLA	Comprehensive Environmental Response, Compensation, and Liability Act
CFR	Code of Federal Regulations
Ci	curie
CI	confidence interval
CLP	Contract Laboratory Program
cm	centimeter
CML	chronic myeloid leukemia
CPSC	Consumer Products Safety Commission
CWA	Clean Water Act
DHEW	Department of Health, Education, and Welfare
DHHS	Department of Health and Human Services
DNA	deoxyribonucleic acid
DOD	Department of Defense
DOE	Department of Energy
DOL	Department of Labor
DOT	Department of Transportation

DOT/UN/ NA/IMDG	Department of Transportation/United Nations/ North America/Intergovernmental Maritime Dangerous Goods Code
DWEL	drinking water exposure level
ECD	electron capture detection
ECG/EKG	electrocardiogram
EEG	electroencephalogram
EEGL	Emergency Exposure Guidance Level
EPA	Environmental Protection Agency
F	Fahrenheit
F_1	first-filial generation
FAO	Food and Agricultural Organization of the United Nations
FDA	Food and Drug Administration
FEMA	Federal Emergency Management Agency
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
FPD	flame photometric detection
fpm	feet per minute
FR	Federal Register
FSH	follicle stimulating hormone
g	gram
GC	gas chromatography
gd	gestational day
GLC	gas liquid chromatography
GPC	gel permeation chromatography
HPLC	high-performance liquid chromatography
HRGC	high resolution gas chromatography
HSDB	Hazardous Substance Data Bank
IARC	International Agency for Research on Cancer
IDLH	immediately dangerous to life and health
ILO	International Labor Organization
IRIS	Integrated Risk Information System
Kd	adsorption ratio
kg	kilogram
kkg	kilokilogram; 1 kilokilogram is equivalent to 1,000 kilograms and 1 metric ton
K_{oc}	organic carbon partition coefficient
K_{ow}	octanol-water partition coefficient
L	liter
LC	liquid chromatography
LC_{50}	lethal concentration, 50% kill
LC_{Lo}	lethal concentration, low
LD_{50}	lethal dose, 50% kill
LD_{Lo}	lethal dose, low
LDH	lactic dehydrogenase
LH	luteinizing hormone
LOAEL	lowest-observed-adverse-effect level
LSE	Levels of Significant Exposure
LT_{50}	lethal time, 50% kill
m	meter
MA	*trans,trans*-muconic acid
MAL	maximum allowable level
mCi	millicurie
MCL	maximum contaminant level

MCLG	maximum contaminant level goal
MF	modifying factor
MFO	mixed function oxidase
mg	milligram
mL	milliliter
mm	millimeter
mmHg	millimeters of mercury
mmol	millimole
mppcf	millions of particles per cubic foot
MRL	Minimal Risk Level
MS	mass spectrometry
mt	metric ton
NAAQS	National Ambient Air Quality Standard
NAS	National Academy of Science
NATICH	National Air Toxics Information Clearinghouse
NATO	North Atlantic Treaty Organization
NCE	normochromatic erythrocytes
NCEH	National Center for Environmental Health
NCI	National Cancer Institute
ND	not detected
NFPA	National Fire Protection Association
ng	nanogram
NHANES	National Health and Nutrition Examination Survey
NIEHS	National Institute of Environmental Health Sciences
NIOSH	National Institute for Occupational Safety and Health
NIOSHTIC	NIOSH's Computerized Information Retrieval System
NLM	National Library of Medicine
nm	nanometer
nmol	nanomole
NOAEL	no-observed-adverse-effect level
NOES	National Occupational Exposure Survey
NOHS	National Occupational Hazard Survey
NPD	nitrogen phosphorus detection
NPDES	National Pollutant Discharge Elimination System
NPL	National Priorities List
NR	not reported
NRC	National Research Council
NS	not specified
NSPS	New Source Performance Standards
NTIS	National Technical Information Service
NTP	National Toxicology Program
ODW	Office of Drinking Water, EPA
OERR	Office of Emergency and Remedial Response, EPA
OHM/TADS	Oil and Hazardous Materials/Technical Assistance Data System
OPP	Office of Pesticide Programs, EPA
OPPT	Office of Pollution Prevention and Toxics, EPA
OPPTS	Office of Prevention, Pesticides and Toxic Substances, EPA
OR	odds ratio
OSHA	Occupational Safety and Health Administration
OSW	Office of Solid Waste, EPA
OTS	Office of Toxic Substances

OW	Office of Water
OWRS	Office of Water Regulations and Standards, EPA
PAH	polycyclic aromatic hydrocarbon
PBPD	physiologically based pharmacodynamic
PBPK	physiologically based pharmacokinetic
PCE	polychromatic erythrocytes
PEL	permissible exposure limit
PEL-C	permissible exposure limit-ceiling value
pg	picogram
PHS	Public Health Service
PID	photo ionization detector
pmol	picomole
PMR	proportionate mortality ratio
ppb	parts per billion
ppm	parts per million
ppt	parts per trillion
PSNS	pretreatment standards for new sources
RBC	red blood cell
REL	recommended exposure level/limit
REL-C	recommended exposure level-ceiling value
RfC	reference concentration (inhalation)
RfD	reference dose (oral)
RNA	ribonucleic acid
RQ	reportable quantity
RTECS	Registry of Toxic Effects of Chemical Substances
SARA	Superfund Amendments and Reauthorization Act
SCE	sister chromatid exchange
SGOT	serum glutamic oxaloacetic transaminase (same as aspartate aminotransferase or AST)
SGPT	serum glutamic pyruvic transaminase (same as alanine aminotransferase or ALT)
SIC	standard industrial classification
SIM	selected ion monitoring
SMCL	secondary maximum contaminant level
SMR	standardized mortality ratio
SNARL	suggested no adverse response level
SPEGL	Short-Term Public Emergency Guidance Level
STEL	short term exposure limit
STORET	Storage and Retrieval
TD_{50}	toxic dose, 50% specific toxic effect
TLV	threshold limit value
TLV-C	threshold limit value-ceiling value
TOC	total organic carbon
TPQ	threshold planning quantity
TRI	Toxics Release Inventory
TSCA	Toxic Substances Control Act
TWA	time-weighted average
UF	uncertainty factor
U.S.	United States
USDA	United States Department of Agriculture
USGS	United States Geological Survey
VOC	volatile organic compound
WBC	white blood cell

WHO	World Health Organization
>	greater than
≥	greater than or equal to
=	equal to
<	less than
≤	less than or equal to
%	percent
α	alpha
β	beta
γ	gamma
δ	delta
μm	micrometer
μg	microgram
q_1^*	cancer slope factor
–	negative
+	positive
(+)	weakly positive result
(–)	weakly negative result